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PROVISIONAL SPECIFICATION

for the invention entitled:

"Viral variants; detection and application"

The invention is described in the following statement:

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VIRAL VARIANTS; DETECTION AND APPLICATION

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates generally to viral variants exhibiting reduced sensitivity to particular agents and/or reduced interactivity with immunological reagents. More particularly, the present invention is directed to hepatitis B virus (HBV) variants exhibiting complete or partial resistance to nucleoside or nucleotide analogs and/or reduced interactivity with antibodies to viral surface components including reduced sensitivity to these antibodies. The present invention further contemplates assays for detecting such viral variants, which assays are useful in monitoring anti-viral therapeutic regimens and in developing new or modified vaccines directed against viral agents and in particular HBV variants. The present invention also contemplates the use of the viral variants to screen for and/or develop or design agents capable of inhibiting infection, replication and/or release of the virus.

DESCRIPTION OF THE PRIOR ART

Bibliographic details of the publications referred to in this specification are also collected at the end of the description.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in any country.

Hepatitis B virus (HBV) can cause debilitating disease conditions and can lead to acute liver failure. HBV is a DNA virus which replicates *via* an RNA intermediate and utilizes reverse transcription in its replication strategy (Summers and Mason, *Cell 29:* 403-415, 1982). The HBV genome is of a complex nature having a partially double-stranded DNA

structure with overlapping open reading frames encoding surface, core, polymerase and X genes. The complex nature of the HBV genome is represented in Figure 1. The polymerase consists of four functional regions, the terminal protein (TP), spacer, reverse transcriptase (rt) and ribonuclease (RNAse).

The polymerase gene of HBV overlaps the envelope gene, mutations in the catalytic domain of the polymerase gene can also affect the nucleotide and the deduced amino acid sequence of the envelope protein and *vice versa*. In particular, the genetic sequence for the neutralization domain of HBV known as the 'a' determinant, which is found within the HBsAg and located between amino acids 99 and 169, actually overlaps the major catalytic regions of the viral polymerase protein and in particular domains A and B.

The presence of an HBV DNA polymerase has led to the proposition that nucleoside or nucleotide analogs could act as effective anti-viral agents. Examples of nucleoside analogs currently being tested are penciclovir and its oral form (FCV) [Vere Hodge, *Antiviral Chem Chemother 4:* 67-84, 1993; Boyd *et al., Antiviral Chem Chemother. 32:* 358-363, 1987; Kruger *et al., Hepatology 22:* 219A, 1994; Main *et al., J. Viral Hepatitis 3:* 211-215, 1996], Lamivudine [(-)-β-2'-deoxy-3'-thiacytidine]; (3TC or LMV) [Severini *et al., Antimicrobial Agents Chemother. 39:* 430-435, 1995; Dienstag *et al., New England J Med 333:* 1657-1661, 1995]. New nucleoside or nucleotide analogs which have already progressed to clinical trials include the pyrimidines Emtricitabine, ((-)-β-L-2'-3'-dideoxy-5-fluoro-3'-thiacydidine; FTC), the 5-fluoro derivative of 3TC, and Clevudine (1-(2-fluoro-5-methyl-β-L-arabino-furanosyl) uracil; L-FMAU), a thymidine analog. Like 3TC, these are pyrimidine derivatives with an unnatural "L"- configuration. Several purine derivatives have also progressed to clinical trials; they include Entecavir (BMS-200, 475; ETV), a carbocyclic deoxyguanosine analog, diaminopurine dioxolane (DAPD), an oral pro-drug for dioxolane guanine ((-)-β-D-2-aminopurine dioxolane; DXG) and Adefovir dipivoxil, an oral prodrug for the acyclic deoxyadenosine monophosphate nucleoside analog Adefovir (9-[phosphonyl-methoxyethyl]-adenine; PMEA). Other drugs in pre-clinical and clinical trials include FLG [Medivir], ACH-126,443 (L-d4C) [Archillion Pharmaceuticals], ICN 2001-3 (ICN) and Racivir (RCV) [Pharmassett].

Whilst these agents are highly effective in inhibiting HBV DNA synthesis, there is the potential for resistant mutants of HBV to emerge during long term antiviral chemotherapy. In patients on prolonged LMV therapy, key resistance mutations are selected in the rt domain within the polymerase at rtM204I/V +/- rtL180M as well as other mutations. The nomenclature used for the polymerase mutations is in accordance with that proposed by Stuyver *et al.*, 2001, *supra*. LMV is a nucleoside analog that has been approved for use against chronic HBV infection. LMV is a particularly potent inhibitor of HBV replication and reduces HBV DNA titres in the sera of chronically infected patients after orthotopic liver transplantation (OLT) by inhibiting viral DNA synthesis. LMV monotherapy seems unlikely to be able to control HBV replication in the longer term. This is because emergence of LMV-resistant strains of HBV seems almost inevitable during monotherapy.

Adefovir dipivoxil (ADV: formerly, bis-pom PMEA) is an orally available prodrug of the acyclic deoxyadenosine monophosphate analog adefovir (formerly, PMEA) (Figure 2). ADV is also a potent inhibitor of HBV replication and has recently been given FDA approval for use against chronic HBV infection. Adefovir dipivoxil differs from other agents in this class in that it is a nucleotide (*vs.* nucleoside) analog and as such bypasses the first phosphorylation reaction during drug activation. This step is often rate-limiting. Adefovir dipivoxil has demonstrated clinical activity against both wild-type and lamivudine-resistant strains of HBV and is currently in phase III clinical Testing (Gilson *et al., J Viral Hepat 6:* 387-395, 1999; Perrillo *et al., Hepatology 32:* 129-134, 2000; Peters *et al., Transplantation 68:* 1912-1914, 1999; Benhamou *et al., Lancet 358:* 718-723, 2001). During phase II studies a 30 mg daily dose of adefovir dipivoxil resulted in a mean 4 $\log_{10}$ decrease in viremia over 12 weeks (Heathcote *et al., Hepatology 28:* A620, 1998).

ADV is a substituted acyclic nucleoside phosphonate. This class of compounds also includes tenofovir disoproxil fumarate (also referred to as tenofovir DF, or tenofovir, or (TFV) or 9-R-(2-phosphonomethoxypropyl)adenine (PMPA) and is marketed as Viread by Gilead sciences).

TFV has antiviral activity against both HBV and HIV (Ying *et al., J Viral Hepat. 7(2):* 161-165, 2000; Ying *et al., J. Viral Hepat. 7(1):* 79-83, 2000; Suo *et al., J Biol Chem. 273(42):* 27250-27258. 1998).

FTC has activity against HBV and HIV (Frick *et al., Antimicrob Agents Chemother 37:* 2285-2292, 1993).

Nucleoside or nucleotide analog therapy may be administered as monotherapy or combination therapy where two or more nucleoside or nucleotide analogs may be administered. The nucleoside or nucleotide analogs may also be administered in combination with other antiviral agents such as interferon or hepatitis B immunoglobulin (HBIG).

There is a need to monitor for the emergence of nucleoside/nucleotide-analog- or antibody-resistant strains of HBV and to develop diagnostic protocols to detect these resistant viruses and/or to use them to screen for and/or develop or design agents having properties making them useful as anti-viral agents. Defective forms of these resistant strains or antigenic components therefrom are also proposed to be useful in the development of therapeutic vaccine compositions as are antibodies directed to viral surface components.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A summary of the sequence identifiers is provided in Table 1. A sequence listing is provided after the claims.

Specific mutations in an amino acid sequence are represented herein as "Xaa_1nXaa_2" where Xaa_1 is the original amino acid residue before mutation, n is the residue number and Xaa_2 is the mutant amino acid. The abbreviation "Xaa" may be the three letter or single letter (i.e. "X") code. An "rt" before "Xaa_1nXaa_2" means "reverse transcriptase". An "s" means an envelope gene. The amino acid residues for HBV DNA polymerase are numbered with the residue methionine in the motif Tyr Met Asp Asp (YMDD) being residue number 204 (Stuyver *et al., Hepatology 33:* 751-757, 2001). The amino acid residues for hepatitis B virus surface antigen are number according to Norder *et al.* (*J. Gen. Virol. 74:* 341-1348, 1993). Both single and three letter abbreviations are used to define amino acid residues and these are summarized in Table 2.

In accordance with the present invention, the selection of HBV variants is identified in patients (Patient A to L) with chronic HBV infection treated with ADV. Patient E is a nonresponder to ADV. Variants of HBV are identified during ADV or combination ADV and LMV treatment with mutations in the HBV DNA polymerase gene which reduce the sensitivity of HBV to this nucleoside analog. Consequently, HBV rt variants are contemplated which are resistant to, or which exhibit reduced sensitivity to, ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and

FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof . Corresponding mutations in the surface antigen also occur. The identification of these HBV variants is important for the development of assays to monitor ADV, LMV, FTC and/or TFV resistance and/or resistance to other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof and to screen for agents which are useful as alternative therapeutic agents.

Reference herein to "anti-HBV agents" includes nucleoside and nucleotide analogs as well as immunological reagents (e.g. antibodies to HBV surface components) and chemical, proteinaceous and nucleic acid agents which inhibit or otherwise interfere with viral replication, maintenance, infection, assembly or release.

The detection of such HBV variants is particularly important in the management of therapeutic protocols including the selection of appropriate agents for treating HBV infection. The method of this aspect of the present invention is predicated in part on monitoring the development in a subject of an increased HBV load in the presence of a nucleoside or nucleotide analog or other anti-HBV agents or combinations thereof. The clinician is then able to modify an existing treatment protocol or select an appropriate treatment protocol accordingly.

Accordingly, one aspect of the present invention is directed to an isolated HBV variant comprising a nucleotide mutation in a gene encoding a DNA polymerase resulting in at least one amino acid addition, substitution and/or deletion to the DNA polymerase and which exhibits decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof. The variant HBV comprises a mutation in an overlapping open reading frame in its genome in a region defined by one or more of domains F and G and domain A through to E of HBV DNA polymerase.

Another aspect of the present invention provides an isolated HBV variant comprising a nucleotide mutation in the S gene resulting in at least one amino acid addition, substitution and/or deletion to the surface antigen and which exhibits decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combinations thereof.

Useful mutants in the rt region include, in one embodiment, rtT38K, rtR55H and rtA181V; in another embodiment includes rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment includes rtN238T; and yet another embodiment includes rtI122V and rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment includes rtT128N and rtN236T, in yet another embodiment includes tL180M, rtM204V rtQ215S, in yet another embodiment includes rtT128S rtL180M, rtM204Vand rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment includes rtN238T/A, in yet another embodiment includes rtI187V, or a combination thereof or an equivalent mutation.

Other HBV variants are also contemplated with mutations rtT38K (in the F domain of the DNA polymerase), rtR55H (located between the F and A domains), rtS/T78S, rtV80L (these are located within the A domain), rtN/S118N, rtI122V rtN/K139K, rtE142V (located between the A and B domains, rtA181V, rtA181T (these are located in the B domain),; rtI187V (located between the B and C domains), rtA/V200V (Located in the C Domain), rtV214A rtQ/P/S/Stop215S, rtQ215S, rt E/K218E (located between the C and D domains), rtn236T, rtH237H/P rtN/H238H, rtN238T, rtN238T/A (these are located in the D domain), rtY245H (located between the D and E domains), and rtV253G (located in the E Domain) or a combination thereof or an equivalent mutation.

Useful mutations in the S gene include, in one embodiment include sQ30K. sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, or a combination thereof or an equivalent mutation.

The present invention further contemplates a method for determining the potential for an HBV to exhibit reduced sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof by isolating DNA or corresponding mRNA from the HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and G and domains A through to E or a region proximal thereto of the DNA polymerase and associated with resistance or decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. The presence of such a mutation is an indication of the likelihood of resistance to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and

FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analògs or other anti-HBV agents or combination thereof.

The present invention also provides a composition comprising a variant HBV resistant to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV, ADV and LMV and FTC and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof or an HBV surface antigen from the variant HBV or a recombinant or derivative form thereof or its chemical equivalent and one or more pharmaceutically acceptable carriers and/or diluents.

Yet another aspect of the present invention provides a use of the aforementioned composition or a variant HBV comprising a nucleotide mutation in a gene encoding a DNA polymerase resulting in at least one amino acid addition, substitution and/or deletion to the DNA polymerase and a decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof in the manufacture of a medicament for the treatment and/or prophylaxis of hepatitis B virus infection.

The present invention also contemplates a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other anti-HBV agents or by isolating DNA or corresponding mRNA from the HBV and screening for a mutation in the nucleotide sequence encoding the DNA polymerase wherein the presence of the following mutations in the rt region: in one embodiment, rtT38K, rtR55H and rtA181V; in another embodiment includes rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment includes rtN238T; and yet another embodiment includes rtI122V and

rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment includes rtT128N and rtN236T, in yet another embodiment includes tL180M, rtM204V rtQ215S, in yet another embodiment includes rtT128S rtL180M, rtM204Vand rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment includes rtN238T/A, in yet another embodiment includes rtI187V, or combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

Still a further methodology comprises screening for a mutation in the nucleotide sequence encoding the envelope genes (s) wherein the presence of the following mutations in the S gene: , in one embodiment include sQ30K. sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, or combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV, and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

Preferably, the variants are in an isolated form such that they have undergone at least one purification step away from naturally occurring body fluid. Alternatively, the variants may be maintained in isolated body fluid or may be in DNA form. The present invention also contemplates infectious molecular clones comprising the genome or parts thereof from a variant HBV. The detection of HBV or its components in cells, cell lysates, cultured supernatant fluid and bodily fluid may be by any convenient means including any nucleic acid-based detection means, for example, by nucleic acid hybridization techniques or *via* one or more polymerase chain reactions (PCRs). The term "bodily fluid" includes any fluid derived from the blood, lymph, tissue or organ systems including serum, whole blood, biopsy and biopsy fluid, organ explants and organ suspension such as liver suspensions.

Another aspect of the present invention is directed to a variant HBV comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to a surface antigen from a reference or wild type HBV and wherein an antibody generated to the reference or wild type surface antigen exhibits an altered immunological profile relative to the HBV variant. One altered profile includes a reduced capacity for neutralizing the HBV. More particularly, the surface antigen of the variant HBV exhibits an altered immunological profile compared to a pre-treatment HBV where the variant HBV is selected for by a nucleoside or nucleotide analog or other anti-HBV agents of the HBV DNA polymerase. The variant HBV of this aspect of the invention may also comprise a nucleotide sequence comprising a single or multiple nucleotide substitution, addition and/or deletion compared to a pre-treatment HBV.

The present invention extends to an isolated HBsAg or a recombinant form thereof or derivative or chemical equivalent thereof corresponding to the variant HBV. Generally, the HBsAg or its recombinant or derivative form or its chemical equivalent comprises an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to an HBsAg from a reference HBV and wherein an antibody directed to a reference HBV exhibits an altered immunological profile to an HBV

carrying said variant HBsAg. In one embodiment, the altered immunological profile comprises a reduction in the ability to neutralize the variant HBV.

Another aspect of the present invention contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV by generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in a plasmid vector and then transfecting said cells with said construct, contacting the cells, before, during and/or after transfection, with the agent to be tested, culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agents; and the subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent. In a preferred embodiment, the plasmid vector in a baculovirus vector and the method comprises generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in or fused to an amount of a baculovirus genome effective to infect cells and then infecting said cells with said construct, contacting the cells, before, during and/or after infection, with the agent to be tested, culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent and then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

In connection with these methods, the plasmid vector may include genes encoding part or all of other viral vectors such as baculovirus vectors or adenovirus vectors (see Ren and Nassal, *J. Virol. 75(3):* 1104-1116, 2001).

In an alternative embodiment, the method comprises generating a continuous cell line comprising an infectious copy of the genome of the HBV in a replication competent

effective amount such that said infectious HBV genome is stably integrated into said continuous cell line such as but not limited to the 2.2.15 or AD cell line, contacting the cells with the agent to be tested, culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to the agent and then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

In an alternative embodiment, the present invention also contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV polymerase in an *in vitro* polymerase assay. The HBV polymerase activity can be examined using established assays (Gaillard *et al., Antimicrob Agents Chemother. 46(4)*: 1005-1013, 2002; Xiong *et al., Hepatology. 28(6)*: 1669-73, 1998). The HBV polymerase may be a wild-type or reference HBV polymerase or mutant HBV polymerase.

The identification of viral variants enables the production of vaccines comprising particular recombinant viral components such as polymerases or envelope genes PreS1, PreS2, S encoding for L, M, S proteins as well as therapeutic vaccines comprising defective HBV variants. Rational drug design may also be employed to identify or generate therapeutic molecules capable of interacting with a polymerase or envelope genes PreS1, PreS2, S encoding for L, M, S proteins or other component of the HBV. Such drugs may also have diagnostic potential. In addition, defective HBV variants may also be used as therapeutic compositions to generate an immune response against the same, similar or homologous viruses. Alternatively, antibodies generated to the HBV variants or surface components thereof may be used in passive immunization of subjects against infection by HBV variants or similar or homologous viruses. Furthermore, agents such as nucleoside or nucleotide analogs, RNAi or siRNA molecules, antisense or sense oligonucleotides, chemical or proteinaceous molecules having an ability to down-regulate the activity of a component of HBV and inhibit replication, maintenance, infection, assembly or release are contemplated by the present invention.

A summary of the abbreviations used throughout the subject specification are provided in Table 3.

A summary of sequence identifiers used throughout the subject specification is provided in Table 1.

TABLE 1

Summary of sequence identifiers

SEQUENCE ID NO:	DESCRIPTION
1	Formula I
2	Formula II
3	OS1 primer
4	TTA3 primer
5	JM primer
6	TTA4 primer
7	OS2 primer
8	sense primer
9	antisense primer
10	internal regions primer
11	internal regions primer
12	PC1 forward primer
13	PC2 reverse primer
14	HBV-specific molecular beacon primer

TABLE 2

Single and three letter amino acid abbreviations

Amino Acid	Three-letter Abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	The	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

TABLE 3

Abbreviations

ABBREVIATION	DESCRIPTION
3TC	(LMV); (-)-β-2'-deoxy-3'-thiacytidine
ADV	adefovir dipivoxil
DAPD	diaminopurine dioxalone
DXG	dioxolane guanine
ETV	entecavir
FAM	famciclovir
FCV	famciclovir
FTC	emtricitabine
HBIG	hepatitis B immunoglobulin
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
LMV	lamividuine
PMEA	9-[phosphonyl-methoxyethyl]-adenine; adefovir
PMPA	9-R-(2-phosphonomethoxypropyl)adenine
RNase	ribonuclease
rt ("rt" before "Xaa_1nXaa_2" means reverse transcriptase)	reverse transcriptase
s (as used in a mutation, e.g. sF134V)	envelope gene
TFV	tenofovir disoproxil fumarate
YMDD	Tyr Met Asp Asp-a motif in the polymerase protein; where the Met residue is designated residue number 204 of the reverse transcriptase

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation showing the partially double stranded DNA HBV genome showing the overlapping open reading frames encoding surface (S), core (C), polymerase (P) and X gene.

Figure 2 is a diagrammatic representation of the chemical structure of ADV.

Figure 3 is a diagrammatic representation of a computer system for determining the value of a variant HBV.

Figure 4 is a representation showing comparison of the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in sequential samples from Patient A during ADV treatment.

Figure 5 is a representation showing comparison of the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient A during ADV therapy.

Figure 6 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient A during ADV therapy.

Figure 7 is a representation showing comparison of the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in sequential samples from Patient B during ADV and LMV treatment.

Figure 8 is a representation showing comparison of the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient B during ADV and LMV therapy.

Figure 9 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient B during ADV and LMV therapy.

Figure 10 is a representation the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient C during ADV treatment.

Figure 11 is a representation the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient C during ADV therapy.

Figure 12 is a representation the deduced amino acid sequence of the envelope gene in samples from Patient C during ADV therapy.

Figure 13 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient D during ADV treatment.

Figure 14 is a representation the deduced amino acid sequence of the catalytic region of the polymerase gene in sequential samples from Patient D during ADV therapy.

Figure 15 is a representation showing comparison of the deduced amino acid sequence of the envelope gene in sequential samples from Patient D during ADV therapy.

Figure 16 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient E during ADV treatment.

Figure 17 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient E during ADV therapy.

Figure 18 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient E during ADV therapy.

Figure 19 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient F during ADV treatment.

Figure 20 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient F during ADV therapy.

Figure 21 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient F during ADV therapy.

Figure 22 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient G during ADV treatment.

Figure 23 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient G during ADV therapy.

Figure 24 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient G during ADV therapy.

Figure 25 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient H during ADV treatment.

Figure 26 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient H during ADV therapy.

Figure 27 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient H during ADV therapy.

Figure 28 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient I during ADV treatment.

Figure 29 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient I during ADV therapy.

Figure 30 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient I during ADV therapy.

Figure 31 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient J during ADV treatment.

Figure 32 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient J during ADV therapy.

Figure 33 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient J during ADV therapy.

Figure 34 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient K during ADV treatment.

Figure 35 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient K during ADV therapy.

Figure 36 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient K during ADV therapy.

Figure 37 is a representation showing the HBV nucleotide sequence encoding the catalytic region of the polymerase gene in samples from Patient L during ADV treatment.

Figure 38 is a representation showing the deduced amino acid sequence of the catalytic region of the polymerase gene in samples from Patient L during ADV therapy.

Figure 39 is a representation showing the deduced amino acid sequence of the envelope gene in samples from Patient L during ADV therapy..

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated in part on the identification and isolation of nucleoside or nucleotide analog-resistant variants of HBV following treatment of patients with either ADV or LMV or more particularly ADV and LMV, or optionally other nucleoside analogs or nucleotide analogs or other anti-HBV agents such as TFV or FTC. In particular, ADV or ADV and LMV treated patients gave rise to variants of HBV exhibiting decreased or reduced sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV . Reference herein to "decreased" or "reduced" in relation to sensitivity to ADV and/or LMV and/or FTC and/or TFV includes and encompasses a complete or substantial resistance to the nucleoside or nucleotide analog or other anti-HBV agents as well as partial resistance and includes a replication rate or replication efficiency which is more than a wild-type in the presence of a nucleoside or nucleotide analog or other anti-HBV agents. In one aspect, this is conveniently measured by an increase in viral load during treatment, or alternatively, there is no substantial decrease in HBV DNA viral load from pre-treatment HBV DNA levels during treatment (i.e., non-response to treatment).

Before describing the present invention in detail, it is to be understood that unless otherwise indicated, the subject invention is not limited to specific formulations of components, manufacturing methods, dosage regimens, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a nucleoside or nucleotide analog" includes a single analog, as well as two or more analogs; reference to "an HBV variant" includes reference to two or more HBV variants; and so forth.

In describing and claiming the present invention, the following terminology is used in accordance with the definitions set forth below.

The terms "analog", "compound", "active agent", "pharmacologically active agent", "medicament", "active" and "drug" are used interchangeably herein to refer to a chemical compound that induces a desired effect such as inhibit viral replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. The terms also encompass pharmaceutically acceptable and pharmacologically active ingredients of those active agents specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the terms "analog", "compound", "active agent", "pharmacologically active agent", "medicament", "active" and "drug" are used, then it is to be understood that this includes the active agent *per se* as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, metabolites, analogs, etc.

The present invention contemplates, therefore, compounds useful in inhibiting HBV replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. Reference to an "analog", "compound", "active agent", "pharmacologically active agent", "medicament", "active" and "drug" such as ADV, LMV, FTC and/or TFV includes combinations of two or more actives such as ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV. A "combination" also includes a two-part or more such as a multi-part anti-HBV therapeutic composition where the agents are provided separately and given or dispensed separately or admixed together prior to dispensation.

The terms "effective amount" and "therapeutically effective amount" of an agent as used herein mean a sufficient amount of the agent to provide the desired therapeutic or physiological effect of inhibiting HBV replication, infection, maintenance, assembly

and/or the function of an enzyme such as HBV DNA polymerase. Furthermore, an "effective HBV-inhibiting amount" or "effective symptom-ameloriating amount" of an agent is a sufficient amount of the agent to directly or indirectly inhibit replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what is an appropriate "effective amount". The exact amount required will vary from subject to subject, depending on the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

By "pharmaceutically acceptable" carrier, excipient or diluent is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e. the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, coloring agents, wetting or emusifying agents, pH buffering agents, preservatives, and the like.

Similarly, a "pharmacologically acceptable" salt, ester, emide, prodrug or derivative of a compound as provided herein is a salt, ester, amide, prodrug or derivative that this not biologically or otherwise undesirable.

The terms "treating" and "treatment" as used herein refer to reduction in severity and/or frequency of symptoms, elimination of symptoms and/or underlying cause, prevention of the occurrence of symptoms and/or their underlying cause, and improvement or remediation of damage in relation to HBV infection. Thus, for example, "treating" a patient involves prevention of HBV infection as well as treatment of a clinically HBV symptomatic individual by inhibiting HBV replication, infection, maintenance, assembly and/or the function of an enzyme such as HBV DNA polymerase. Thus, for example, the

present method of "treating" a patient with HBV infection or with a propensity for one to develop encompasses both prevention of HBV infection as well as treating HBV infection or symptoms thereof. In any event, the present invention contemplates the treatment or prophylaxis of HBV infection.

"Patient" as used herein refers to an animal, preferably a mammal and more preferably a primate including a lower primate and even more preferably, a human who can benefit from the formulations and methods of the present invention. A patient regardless of whether a human or non-human animal may be referred to as an individual, subject, animal, host or recipient. The compounds and methods of the present invention have applications in human medicine, veterinary medicine as well as in general, domestic or wild animal husbandry. For convenience, an "animal" includes an avian species such as a poultry bird (including ducks, chicken, turkeys and geese), an aviary bird or game bird. The condition in a non-human animal may not be a naturally occurring HBV infection but HBV-like infection may be induced.

As indicated above, the preferred animals are humans, non-human primates such as marmossets, baboons, orangatangs, lower primates such as tupia, livestock animals, laboratory test animals, companion animals or captive wild animals. A human is the most preferred target. However, non-human animal models may be used.

Examples of laboratory test animals include mice, rats, rabbits, guinea pigs and hamsters. Rabbits and rodent animals, such as rats and mice, provide a convenient test system or animal model as do primates and lower primates. Livestock animals include sheep, cows, pigs, goats, horses and donkeys. Non-mammalian animals such as avian species, zebrafish, amphibians (including cane toads) and *Drosophila* species such as *Drosophila melanogaster* are also contemplated. Instead of a live animal model, a test system may also comprise a tissue culture system.

Accordingly, one aspect of the present invention is directed to an isolated Hepatitis B virus (HBV) variant wherein said variant comprises a nucleotide mutation in a gene encoding a

DNA polymerase resulting in at least one amino acid addition, substitution and/or deletion to said DNA polymerase and wherein said variant exhibits decreased sensitivity to one of ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV or optionally other nucleoside analogs or other anti-HBV agents or a combination thereof.

An "anti-HBV agent" includes a nucleoside or nucleotide analog, protein, chemical compound, RNA or DNA or RNAi or siRNA oligonucleotide.

Preferably, the decreased sensitivity is in respect of ADV. Alternatively, the decreased sensitivity is in respect of LMV. Alternatively, the decreased sensitivity is in respect of TFV. Alternatively, the decreased sensitivity is in respect of FTC. Alternatively, the decreased sensitivity is in respect of ADV and LMV. Alternatively, the decreased sensitivity is in respect of ADV and TFV. Alternatively, the decreased sensitivity is in respect of LMV and TFV. Alternatively, the decreased sensitivity is in respect of ADV and FTC. Alternatively, the decreased sensitivity is in respect to FTC and TFV. Alternatively, the decreased sensitivity is in respect of FTC and LMV. Alternatively, the decreased sensitivity is in respect of ADV and LMV and TFV. Alternatively, the decreased sensitivity is in respect to ADV and TFV and FTC. Alternatively, the decreased sensitivity is in respect to LMV and TFV and FTC. Alternatively, the decrease sensitivity is in respect of ADV and LMV and FTC. Alternatively, the decreased sensitivity is in respect of ADV and FTC and TFV and LMV.

Reference herein to "anti-HBV agents" includes nucleoside and nucleotide analogs as well as immunological reagents (e.g. antibodies to HBV surface components) and chemical, proteinaceous and nucleic acid agents which inhibit or otherwise interfere with viral replication, maintenance, infection, assembly or release. Reference herein to "nucleic acid" includes reference to a sense or antisense molecule, RNA or DNA, oligonucleotides and RNAi and siRNA molecules and complexes containing same.

In addition to a mutation in the gene encoding DNA polymerase, due to the overlapping nature of the HBV genome (Figure 1), a corresponding mutation may also occur in the gene encoding the S gene encoding the surface antigen (HBsAg) resulting in reduced interactivity of immunological reagents such as antibodies and immune cells to HBsAg. The reduction in the interactivity of immunological reagents to a viral surface component generally includes the absence of immunological memory to recognize or substantially recognize the viral surface component. The present invention extends, therefore, to an HBV variant exhibiting decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV or a reduced interactivity of an immunological reagent to HBsAg wherein the variant is selected for following ADV and/or LMV combination or sequential treatment. The term "sequential" in this respect means ADV followed by LMV and/or TFV, and /or FTC, LMV followed by ADV and/or TFV, and /or FTC, or multiple sequential administrations of each of ADV, LMV and/or TFV, and /or FTC.

A viral variant may, therefore, carry mutation only in the DNA polymerase gene or both in the DNA polymerase gene and the S gene. The term "mutation" is to be read in its broadest context and includes multiple mutations.

The present invention extends to a mutation and any domain of the HBV DNA polymerase and in particular regions F and G, and domains A through to E provided said mutation leads to decreased sensitivity to ADV and/ or LMV and/or TFV or combinations thereof. Regions F and G of the HBV DNA polymerase is defined by the amino acid sequence set forth in Formula I below [SEQ ID NO:1]:

FORMULA I

L, B_1, B_2, D, W, G, P, C, B_3, B_4, H, G, B_5, H, B_6, I, R, B_7, P, R, T, P, B_8, R, V, B_9, G, G, V, F, L, V, D, K, N, P, H, N, T, B_{10}, E, S, B_{11}, L, B_{12}, V, D, F, S, Q, F, S, R, G, B_{13}, B_{14}, B_{15}, V, S, W, P, K, F, A, V, P, N, L, B_{16}, S, L, T, N, L, L, Sx

wherein:

B_1	is L, or R, or I
B_2	is E, or D
B_3	is T, or D, or A, or N, or Y
B_4	is E, or D
B_5	is E, or K, or Q
B_6	is H, or R, or N,
B_7	is I, or T
B_8	is A, or S
B_9	is T or R
B_{10}	is A, or T, or S
B_{11}	is R, or T
B_{12}	is V, or G
B_{13}	is S, or I, or T, or N, or V
B_{14}	is T, or S, or H, or Y
B_{15}	is R, or H, or K, or Q
B_{16}	is Q, or P;

and wherein Sx is designated as amino acid 74.

In this specification, reference is particularly made to the conserved regions of the DNA polymerase as defined by domains A to E. Regions A to E are defined by the amino acid sequence set forth in Formula II below [SEQ ID NO:2] (and in Australian Patent No. 734831):

FORMULA II

S Z_1 L S W L S L D V S A A F Y H Z_2 P L H P A A M P H L L Z_3 G S S G L Z_4 R Y V A R L S S Z_5 S Z_6 Z_7 X N Z_8 Q Z_9 Z_{10} X X X Z_{11} L H Z_{12} Z_{13} C S R Z_{14} L Y V S L Z_{15} L L Y Z_{16} T Z_{17} G Z_{18} K L H L Z_{19} Z_{20} H P I Z_{21} L G F R K Z_{22} P M G Z_{23} G L S P F L L A Q F T S A I Z_{24} Z_{25} Z_{26} Z_{27} Z_{28} R A F Z_{29} H C Z_{30} Z_{31} F Z_{32} Y M^x D D Z_{33} V L G A Z_{34} Z_{35} Z_{36} Z_{37} H Z_{38} E Z_{39} L Z_{40} Z_{41} Z_{42} Z_{43} Z_{44} Z_{45} Z_{46} L L Z_{47} Z_{48} G I H L N P Z_{49} K T K R W G Y S L N F M G Y Z_{50} I G

wherein:

X is any amino acid;
Z_1 is N or D;
Z_2 is I or P;
Z_3 is I or V;
Z_4 is S or D;
Z_5 is T or N;
Z_6 is R or N;
Z_7 is N or I;
Z_8 is N or Y or H;
Z_9 is H or Y;
Z_{10} is G or R;
Z_{11} is D or N;
Z_{12} is D or N;
Z_{13} is S or Y;
Z_{14} is N or Q;
Z_{15} is L or M;
Z_{16} is K or Q;
Z_{17} is Y or F;
Z_{18} is R or W;

Z_{19} is Y or L;
Z_{20} is S or A;
Z_{21} is I or V;
Z_{22} is I or L;
Z_{23} is V or G;
Z_{24} is C or L;
Z_{25} is A or S;
Z_{26} is V or M;
Z_{27} is V or T;
Z_{28} is R or C;
Z_{29} is F or P;
Z_{30} is L or V;
Z_{31} is A or V;
Z_{32} is S or A;
Z_{33} is V or L or M;
Z_{34} is K or R;
Z_{35} is S or T;
Z_{36} is V or G;
Z_{37} is Q or E;
Z_{38} is L or S or R;
Z_{39} is S or F;
Z_{40} is F or Y;
Z_{41} is T or A;
Z_{42} is A or S;
Z_{43} is V or I;
Z_{44} is T or C;
Z_{45} is N or S;
Z_{46} is F or V;
Z_{47} is S or D;
Z_{48} is L or V;
Z_{49} is N or Q;

Z_{50} is V or I; and

M^x is amino acid 204;

and wherein the first S is designated as amino acid 75.

Preferably, the mutation results in an altered amino acid sequence in any one or more of domains F and G, and domains A through to E or regions proximal thereto of the HBV DNA polymerase.

Another aspect of the present invention provides an HBV variant comprising a mutation in an overlapping open reading frame in its genome wherein said mutation is in a region defined by one or more of domains F and G, and domains A through to E of HBV DNA polymerase and wherein said variant exhibits decreased sensitivity to ADV, LMV,TFV or FTC; ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; and/or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents.

In a related embodiment, there is provided an HBV variant comprising a mutation in the nucleotide sequence encoding a DNA polymerase resulting in an amino acid addition, substitution and/or deletion in said DNA polymerase in one or more amino acids as set forth in Formula I [SEQ ID NO:1] and/or Formula II [SEQ ID NO:2]:

FORMULA I

L, B_1, B_2, D, W, G, P, C, B_3, B_4, H, G, B_5, H, B_6, I, R, B_7, P, R, T, P, B_8, R, V, B_9, G, G, V, F, L, V, D, K, N, P, H, N, T, B_{10}, E, S, B_{11}, L, B_{12}, V, D, F, S, Q, F, S, R, G, B_{13}, B_{14}, B_{15}, V, S, W, P, K, F, A, V, P, N, L, B_{16}, S, L, T, N, L, L, Sx

wherein:

B_1 is L, or R, or I

B_2 is E, or D

B_3 is T, or D, or A, or N, or Y

B_4 is E, or D

B_5 is E, or K, or Q

B_6 is H, or R, or N,

B_7 is I, or T

B_8 is A, or S

B_9 is T or R

B_{10} is A, or T, or S

B_{11} is R, or T

B_{12} is V, or G

B_{13} is S, or I, or T, or N, or V

B_{14} is T, or S, or H, or Y

B_{15} is R, or H, or K, or Q

B_{16} is Q, or P;

and

FORMULA II

S Z_1 L S W L S L D V S A A F Y H Z_2 P L H P A A M P H L L Z_3 G S S G L Z_4 R Y V A R L S S Z_5 S Z_6 Z_7 X N Z_8 Q Z_9 Z_{10} X X X Z_{11} L H Z_{12} Z_{13} C S R Z_{14} L Y V S L Z_{15} L L Y Z_{16} T Z_{17} G Z_{18} K L H L Z_{19} Z_{20} H P I Z_{21} L G F R K Z_{22} P M G Z_{23} G L S P F L L A Q F T S A I Z_{24} Z_{25} Z_{26} Z_{27} Z_{28} R A F Z_{29} H C Z_{30} Z_{31} F Z_{32} Y M^x D D Z_{33} V L G A Z_{34} Z_{35} Z_{36} Z_{37} H Z_{38} E Z_{39} L Z_{40} Z_{41} Z_{42} Z_{43} Z_{44} Z_{45} Z_{46} L L Z_{47} Z_{48} G I H L N P Z_{49} K T K R W G Y S L N F M G Y Z_{50} I G

wherein:

X is any amino acid;

Z_1 is N or D;

Z_2 is I or P;

Z_3 is I or V;

Z_4 is S or D;

Z_5 is T or N;

Z_6 is R or N;

Z_7 is N or I;

Z_8 is N or Y or H;

Z_9 is H or Y;

Z_{10} is G or R;

Z_{11} is D or N;

Z_{12} is D or N;

Z_{13} is S or Y;

Z_{14} is N or Q;

Z_{15} is L or M;

Z_{16} is K or Q;

Z_{17} is Y or F;

Z_{18} is R or W;

Z_{19} is Y or L;

Z_{20} is S or A;

Z_{21} is I or V;

Z_{22} is I or L;

Z_{23} is V or G;

Z_{24} is C or L;

Z_{25} is A or S;

Z_{26} is V or M;

Z_{27} is V or T;

Z_{28} is R or C;

Z_{29} is F or P;

Z_{30} is L or V;

Z_{31} is A or V;

Z_{32} is S or A;
Z_{33} is V or L or M;
Z_{34} is K or R;
Z_{35} is S or T;
Z_{36} is V or G;
Z_{37} is Q or E;
Z_{38} is L or S or R;
Z_{39} is S or F;
Z_{40} is F or Y;
Z_{41} is T or A;
Z_{42} is A or S;
Z_{43} is V or I;
Z_{44} is T or C;
Z_{45} is N or S;
Z_{46} is F or V;
Z_{47} is S or D;
Z_{48} is L or V;
Z_{49} is N or Q;
Z_{50} is V or I; and
M^x is amino acid 204;

and wherein Sx in Formula I is designated as amino acid 74 and the first S in Formula II is designated as amino acid 75;

and wherein said variant exhibits decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; ADV and LMV and TFV; ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. Preferably, the decreased sensitivity is to ADV or to both ADV and LMV or to one or both of ADV and/or LMV and/or TFV and /or FTC.

Another preferred aspect of the present invention contemplates an HBV variant comprising a mutation in the nucleotide sequence encoding HBsAg resulting in an amino acid addition, substitution and/or deletion in said HBsAg in a region corresponding to the amino acid sequence set forth in Formulae I and II wherein said variant exhibits decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

More particularly, the present invention provides a variant HBV comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to a surface antigen from a reference or wild-type HBV and wherein an antibody generated to the reference or wild-type surface antigen exhibits reduced capacity for neutralizing said HBV variant, said variant selected by exposure of a subject to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

The term "combination therapy" means that both combinations of ADV, LMV, FTC and/or TFV are co-administered in the same composition or simultaneously in separate compositions. The term "sequential therapy" means that the two agents are administered within seconds, minutes, hours, days or weeks of each other and in either order. Sequential therapy also encompasses completing a therapeutic course with one or other of ADV, LMV, FTC or TFV and then completing a second or third or subsequent therapeutic courses with the other of ADV, LMV, FTC or TFV.

Accordingly, another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to LMV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Even yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV and LMV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Even still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

A further aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject

to ADV and FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to TFV and FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to FTC and LMV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Even yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Even still another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile

compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

A further aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV and FTC therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to FTC, LMV and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, FTC and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Still yet another aspect of the present invention contemplates an HBV variant comprising a surface antigen having an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or truncation compared to the pretreatment HBV and

wherein the surface antigen of the variant HBV exhibits an altered immunological profile compared to the pretreatment HBV where the said variant HBV is selected for by exposure of a subject to ADV, LMV, FTC and TFV therapy or therapy by one or more other nucleoside or nucleotide analogs or other anti-HBV agents.

Preferably, the variants are in isolated form such that they have undergone at least one purification step away from naturally occurring body fluid. Alternatively, the variants may be maintained in isolated body fluid or may be in DNA form. The present invention also contemplates infectious molecular clones comprising the genome or parts thereof from a variant HBV. Furthermore, the present invention provides isolated components from the variant HBVs such as but not limited to an isolated HBsAg. Accordingly, the present invention provides an isolated HBsAg or a recombinant form thereof or derivative or chemical equivalent thereof, said HBsAg being from a variant HBV selected by exposure of a subject to one or more of ADV, LMV, FTC and/or TFV or optionally one or more nucleoside or nucleotide analogs or other anti-HBV agents.

More particularly, yet another aspect of the present invention is directed to an isolated variant HBsAg or a recombinant or derivative form thereof or a chemical equivalent thereof wherein said HBsAg or its recombinant or derivative form or its chemical equivalent exhibits an altered immunological profile compared to an HBsAg from a reference HBV, said HBsAg being from a variant HBV selected by exposure of a subject to one or more of ADV, LMV, FTC and/or TFV or optionally one or more nucleoside or nucleotide analogs or other anti-HBV agents.

Even more particularly, the present invention provides an isolated variant HBsAg or a recombinant or derivative form thereof or a chemical equivalent thereof wherein said HBsAg or its recombinant or derivative form or its chemical equivalent comprises an amino acid sequence with a single or multiple amino acid substitution, addition and/or deletion or a truncation compared to an HBsAg from a reference HBV and wherein a neutralizing antibody directed to a reference HBV exhibits no or reduced neutralising activity to an HBV carrying said variant HBsAg, said HBsAg being from a variant HBV

selected by exposure of a subject to one or more of ADV, LMV, FTC and/or TFV or optionally one or more nucleoside or nucleotide analogs or other anti-HBV agents.

Preferred mutations in the HBV DNA polymerase include variants selected from patients with HBV recurrence following ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV treatment. Nucleoside or nucleotide analog or other anti-HBV agents treatment may occur in relation to a transplantation procedure (e.g. bone marrow transplantation (BMT) or OLT) or following treatment of patients diagnosed with hepatitis. Following selection of variants, viral loads are obtainable at levels similar to pre-treatment levels or are increasing while on therapy.

Useful mutants in the rt region include, in one embodiment, rtT38K, rtR55H and rtA181V; in another embodiment includes rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment includes rtN238T; and yet another embodiment includes rtI122V and rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment includes rtT128N and rtN236T, in yet another embodiment includes tL180M, rtM204V rtQ215S, in yet another embodiment includes rtT128S rtL180M, rtM204Vand rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment includes rtN238T/A, in yet another embodiment includes rtI187V, or a combination thereof or an equivalent mutation

Such HBV variants are proposed to exhibit a decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. It should be noted that the nomenclature system for amino acid positions is based on the methionine residues in the YMDD motif being designated codon rtM204. This

numbering system is different to that in Australian Patent No. 734831 where the methionine residue in the YMDD motif within the polymerase gene is designated codon 550. In this regard, rtL180M and rtM204V correspond to L526M and M550V, respectively, in Australian Patent No. 734831. Corresponding mutations may also occur in envelope genes such as in one or more of PreS1, PreS2 and S.

Another potential mode of action of ADV and other acyclic nucleoside phosphonates is that of immune stimulation (Calio *et al., Antiviral Res. 23:* 77-89, 1994). A number of mutations resulted in changes in the envelope gene detected in HBV variants which may be associated with immune escape. These changes include in one embodiment include in one embodiment include sQ30K. sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, or a combination thereof or an equivalent mutation.

The identification of the variants of the present invention permits the generation of a range of assays to detect such variants. The detection of such variants may be important in identifying resistant variants to determine the appropriate form of chemotherapy and/or to monitor vaccination protocols, or develop new or modified vaccine preparations.

Still another aspect of the present invention contemplates a method for determining the potential for an HBV to exhibit reduced sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other

nucleoside or nucleotide analogs or other anti-HBV agents, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and G, and A domains through to E or a region proximal thereto of said DNA polymerase and associated with resistance or decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents wherein the presence of such a mutation is an indication of the likelihood of resistance to said ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents.

Preferably, the assay detects one or more of the following mutations: in one embodiment, rtT38K, rtR55H and rtA181V; in another embodiment includes rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment includes rtN238T; and yet another embodiment includes rtI122V and rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment includes rtT128N and rtN236T, in yet another embodiment includes tL180M, rtM204V rtQ215S, in yet another embodiment includes rtT128S rtL180M, rtM204Vand rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment includes rtN238T/A, in yet another embodiment includes rtI187V; or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and

FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

Accordingly, another aspect of the present invention produces a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other anti-HBV agents, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding the DNA polymerase and/or a corresponding region of the S gene, wherein the presence of a mutation selected from, in one embodiment include sQ30K. sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, and in yet another embodiment include sT47A and sW172stop, in even still another embodiment, rtT38K, rtR55H and rtA181V; in another embodiment includes rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment includes rtN238T; and yet another embodiment includes rtI122V and rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment includes rtT128N and rtN236T, in yet another embodiment includes tL180M, rtM204V rtQ215S, in yet another embodiment includes rtT128S rtL180M, rtM204Vand rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment includes rtN238T/A, in yet another embodiment includes rtI187V, or combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV

and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

A further aspect of the present invention produces a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other anti-HBV agents, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding the DNA polymerase and/or a corresponding region of the S gene, wherein the presence of a mutation selected from, in one embodiment, sQ30K. sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, and in yet another embodiment include sT47A and sW172stop, in even still another embodiment, rtT38K, rtR55H and rtA181V; in another embodiment includes rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment includes rtN238T; and yet another embodiment includes rtI122V and rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment includes rtT128N and rtN236T, in yet another embodiment includes tL180M, rtM204V rtQ215S, in yet another embodiment includes rtT128S rtL180M, rtM204Vand rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment includes rtN238T/A, in yet another embodiment includes rtI187V, combinations thereof or an equivalent one or more other mutation is indicative of a variant which exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC

and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

The detection of HBV or its components in cells, cell lysates, cultured supernatant fluid and bodily fluid may be by any convenient means including any nucleic acid-based detection means, for example, by nucleic acid hybridization techniques or *via* one or more polymerase chain reactions (PCRs). The term "bodily fluid" includes any fluid derived from the blood, lymph, tissue or organ systems including serum, whole blood, biopsy and biopsy fluid, organ explants and organ suspension such as liver suspensions. The invention further encompasses the use of different assay formats of said nucleic acid-based detection means, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), single-strand chain polymorphism (SSCP), amplification and mismatch detection (AMD), interspersed repetitive sequence polymerase chain reaction (IRS-PCR), inverse polymerase chain reaction (iPCR) and reverse transcription polymerase chain reaction (RT-PCR), amongst others. Other forms of detection include Northern blots, Southern blots, PCR sequencing, antibody procedures such as ELISA, Western blot and immunohistochemistry. A particularly useful assay includes the reagents and components required for immobilized oligonucleotide- or oligopeptide-mediated detection systems.

One particularly useful nucleic acid detection system is the reverse hybridization technique. In this technique, DNA from an HBV sample is amplified using a biotin or other ligand-labeled primer to generate a labeled amplicon. Oligonucleotides immobilized to a solid support such as a nitrocellulose film are then used to capture amplified DNA by hybridization. Specific nucleic acid fragments are identified *via* biotin or the ligand. Generally, the labeled primer is specific for a particular nucleotide variation to be detected. Amplification occurs only if the variation to be detected is present. There are many forms of the reverse hybridization assay and all are encompassed by the present invention.

Detecting HBV replication in cell culture is particularly useful.

This and other aspects of the present invention is particularly amenable to microarray analysis such as to identify oligonucleotides including sense and antisense molecules, RNAi or siRNA molecules or DNA or RNA-binding molecules which down-regulate genomic sequences or transcripts of HBV. Microarray analysis may also be used to identify particular mutations in the HBV genome such as within the HBV DNA polymerase-coding region or the HBsAg-coding region.

Another aspect of the present invention contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV by:

generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in a plasmid vector and then transfecting said cells with said construct;

contacting the cells, before, during and/or after transfection, with the agent to be tested;

culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agents; and

then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

In a preferred embodiment, the plasmid vector may include genes encoding part or all of other viral vectors such as baculovirus or adenovirus (Ren and Nassal, 2001, *supra*) and the method comprises:

generating a genetic construct comprising a replication competent-effective amount of the genome from the HBV contained in or fused to an amount of a baculovirus genome or adenovirus genome effective to infect cells and then infecting said cells with said construct;

contacting the cells, before, during and/or after infection, with the agent to be tested;

culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

In an alternative embodiment, the method comprises:

generating a continuous cell line comprising an infectious copy of the genome of the HBV in a replication competent effective amount such that said infectious HBV genome is stably integrated into said continuous cell line such as but not limited to 2.2.15 or AD;

contacting the cells with the agent to be tested;

culturing the cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to the agent; and

then subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of the agent.

The above-mentioned methods are particularly useful in identifying or developing agents against HBV variants such as those carrying mutations, in one embodiment, rtT38K, rtR55H and rtA181V; in another embodiment includes rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment includes rtN238T; and yet another embodiment includes rtI122V and rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment includes rtT128N and rtN236T, in yet another embodiment includes tL180M, rtM204V rtQ215S, in yet another embodiment includes rtT128S rtL180M, rtM204Vand rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment includes rtN238T/A, in yet another embodiment includes rtI187V, or a combination thereof or an equivalent mutation; in a further embodiment, sQ30K. sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, or a combination thereof or an equivalent mutation.

Accordingly, another aspect of the present invention contemplates a method for determining whether an HBV strain exhibits reduced sensitivity to a nucleoside or nucleotide analog or other potential anti-HBV agent, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence of the envelope genes or DNA polymerase gene selected from, in one embodiment, in one embodiment, rtT38K, rtR55H and rtA181V; in another embodiment includes rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment includes rtN238T; and yet another embodiment includes rtI122V and rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment includes rtT128N and rtN236T, in yet another embodiment includes tL180M, rtM204V rtQ215S, in yet another embodiment includes rtT128S rtL180M, rtM204Vand rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment includes rtN238T/A, in yet another embodiment includes rtI187V, or a combination thereof or an equivalent mutation; in a further embodiment, sQ30K. sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, or combinations thereof or an equivalent one or more other mutation is indicative of a variant wherein said variant exhibits a decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof.

The detection of amino acid variants of DNA polymerase is conveniently accomplished by reference to the amino acid sequence shown in Formulae I and II. The polymorphisms shown represent the variations shown in various databases for active pathogenic HBV strains. Where an HBV variant comprises an amino acid different to what is represented, then such an isolate is considered a putative HBV variant having an altered DNA polymerase activity.

The present invention further contemplates agents which inhibit ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV resistant HBV variants. Such agents are particularly useful if long term treatment by ADV, LMV, FTC and/or TFV and/or optionally other nucleoside analogs or nucleotide analogs such as TFV is contemplated by the clinician. The agents may be DNA or RNA or proteinaceous or non-proteinaceous chemical molecules. Natural product screening such as from plants, coral and microorganisms is also contemplated as a useful potential source of masking agents as is the screening of combinatorial or chemical libraries. The agents may be in isolated form or in the form of a pharmaceutical composition or formulation and may be administered in place of or sequentially or simultaneously with a nucleoside or nucleotide analog. Furthermore, rationale drug design is contemplated including solving the crystal or NMR structure of, for example, HBV DNA polymerase and designing agents which can bind to the enzyme's active site. This approach may also be adapted to other HBV components.

Accordingly, another aspect of the present invention contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV which exhibits resistance or decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof, , said method comprising:

generating a genetic construct comprising a replication competent-effective amount of the genome from said HBV contained in a plasmid vector and then transfecting said cells with said construct;

contacting said cells, before, during and/or after transfection, with the agent to be tested;

culturing said cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of said agent.

Still another aspect of the present invention provides a method for detecting an agent which exhibits inhibitory activity to an HBV which exhibits resistance or decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof, , said method comprising:

generating a genetic construct comprising a replication competent-effective amount of the genome from said HBV contained in or fused to an amount of a baculovirus genome effective to infect cells and then infecting said cells with said construct;

contacting said cells, before, during and/or after infection, with the agent to be tested;

culturing said cells for a time and under conditions sufficient for the HBV to replicate, express genetic sequences and/or assemble and/or release virus or virus-like particles if resistant to said agent; and

subjecting the cells, cell lysates or culture supernatant fluid to viral- or viral-component-detection means to determine whether or not the virus has replicated, expressed genetic material and/or assembled and/or been released in the presence of said agent.

Preferably, the HBV genome is stably integrated into the cells' genome.

Particularly useful cells are 2.2.15 cells (Price *et al., Proc. Natl. Acad. Sci. USA 86(21):* 8541-8544, 1989 or AD cells (also known as HepAD32 cells or HepAD79 cells [Ying *et al., Viral Hepat. 7(2):* 161-165, 2000.

Whilst the baculovirus vector is a particularly useful in the practice of the present invention, the subject invention extends to a range of other vectors such as but not limited to adenoviral vectors.

The present invention further extends to cell lines (e.g. 2.2.15 or AD cells) carrying genetic constructs comprising all or a portion of an HBV genome or a gene or part of a gene therefrom.

The present invention also provides for the use of the subject HBV variants to screen for anti-viral agents. These anti-viral agents inhibit the virus. The term "inhibit" includes antagonizing or otherwise preventing infection, replication, assembly and/or release or any intermediate step. Preferred anti-viral agents include nucleoside or nucleotide analogs or anti-HBV agents, however, the present invention extends to non-nucleoside molecules.

In addition, rational drug design is also contemplated to identify or generate chemical molecules which either mimic a nucleoside or which interact with a particular nucleotide sequence or a particular nucleotide. Combinatorial chemistry and two hybrid screening are

some of a number of techniques which can be employed to identify potential therapeutic or diagnostic agents.

In one example, the crystal structure or the NMR structure of polymerase or the surface antigen is used to rationally design small chemical molecules likely to interact with key regions of the molecule required for function and/or antigenicity. Such agents may be useful as inhibitors of polymerase activity and/or may alter an epitope on the surface antigen.

Several models of the HBV polymerase have been prepared due to the similarity with reverse transcriptase from HIV (Das *et al., J. Virol. 75(10):* 4771-4779, 2001; Bartholomeusz *et al., Intervirology 40(5-6):* 337-342 1997; Allen *et al., Hepatology 27(6):* 1670-1677, 1998). The models of the HBV polymerase can be used for the rational drug design of new agents effective against HBV encoding the resistant mutations as well as wild type virus. The rational drug that is designed may be based on a modification of an existing antiviral agent such as the agent used in the selection of the HBV encoding the mutations associated with resistance. Viruses or clones expressing HBV genomic material encoding the mutations may also be used to screen for new antiviral agents.

In an alternative embodiment, the present invention also contemplates a method for detecting an agent which exhibits inhibitory activity to an HBV polymerase in an *in vitro* polymerase assay. The HBV polymerase activity can be examined using established assays (Gaillard *et al., Antimicrob Agents Chemother. 46(4):* 1005-1013, 2002; Xiong *et al., Hepatology 28(6):* 1669-1673, 1998).

As indicated above, microarray technology is also a useful means of identifying agents which are capable of interacting with defined HBV internal or external components. For example, arrays of HBV DNA polymerase or peptide fragments thereof carrying different amino acid variants may be used to screen for agents which are capable of binding or otherwise interacting with these molecules. This is a convenient way of determining the differential binding patterns of agents between HBV variants. Arrays of antibodies may

also be used to screen for altered HBsAg molecules. Microarrays are also useful in proteomic analysis to identify molecules such as antibodies, interferons or cytokines which have an ability to interact with an HBV component. Microarrays of DNA and RNA molecules may also be employed to identify sense and antisense molecules for genetic regions on the HBV genome or transcripts thereof.

The above methods are particularly useful in identifying an inhibitor of an HBV resistant to or exhibiting reduced sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof. The present invention extends, therefore, to compositions of the inhibitors. The inhibitors may also be in the form of antibodies or genetic molecules such as ribozymes, antisense molecules and/or sense molecules for co-suppression or the induction of RNAi or may be other nucleoside or nucleotide analogs or other anti-HBV agents or derivatives of known analogs. Reference to RNAi includes reference to short, interfering RNAs (siRNA).

The term "composition" includes a "pharmaceutical composition" or a formulation.

The inhibitor is referred to below as an "active ingredient" or "active compound" and may be selected from the list of inhibitors given above.

The composition may include an antigenic component of the HBV, a defective HBV variant or an agent identified through natural product screening or rational drug design (including combinatorial chemistry).

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is

incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of encoding an aspartyl protease inhibitor. The vector may, for example, be a viral vector.

Pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) and sterile powders for the extemporaneous preparation of sterile injectable solutions. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dilution medium comprising, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of superfactants. The preventions of the action of microorganisms can be brought about by various anti-bacterial and anti-fungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with the active ingredient and optionally other active ingredients as required, followed by filtered sterilization or other appropriate means of sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, suitable methods of preparation include vacuum drying and the freeze-drying technique which yield a powder of active ingredient plus any additionally desired ingredient.

When the active ingredient is suitably protected, it may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets. For oral therapeutic administration, the active ingredient may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 200 mg of active compound. Alternative dosage amounts include from about 1 μg to about 1000 mg and from about 10 μg to about 500 mg. These dosages may be per individual or per kg body weight. Administration may be per hour, day, week, month or year.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter. A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and a flavouring. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

As stated above, the present invention further extends to an isolated HBsAg from the HBV variants herein described. More particularly, the present invention provides an HBsAg or a recombinant form thereof or derivative or chemical equivalent thereof. The isolated surface component and, more particularly, isolated surface antigen or its recombinant, derivative or chemical equivalents are useful in the development of biological compositions such as vaccine formulations.

Yet another aspect of the present invention provides a composition comprising a variant HBV resistant to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or an HBV surface antigen from said variant HBV or a recombinant or derivative form thereof or its chemical equivalent and one or more pharmaceutically acceptable carriers and/or diluents. Such a composition may be regarded as a therapeutic composition and is useful in generating an immune response including a humoral response. Generally, the HBV variants are "defective" and in themselves are unable to cause a sustained infection in a subject.

As indicated above, antibodies may be generated to the mutant HBV agents and used for passive or direct vaccination against infection by these viruses. The antibodies may be generated in humans or non-human animals. In the case of the latter, the non-human antibodies may need to be deimmunized or more specifically humanized prior to use. Deimmunized may include, for example, grafting complimentarity determining regions (CDRs) from the variable region of a murine or non-human animal anti-HBV antibody onto a human consensus fragment antibody binding (Fab) polypeptide. Alternatively, amino acids defining epitopes in the variable region of the antibody may be mutated so that the epitopes are no longer recognized by the human MHC II complex.

Insofar as ribozyme, antisense or co-suppression (RNAi) or siRNA or complexes thereof repression is concerned, this is conveniently aimed at post-transcription gene silencing.

DNA or RNA may be administered or a complex comprising RNAi or a chemical analog thereof specific for HBV mRNA may be employed.

All such molecules may be incorporated into pharmaceutical compositions.

In another embodiment, the present invention provides a biological composition comprising a variant HBV or an HBsAg or L, M or S proteins from said variant HBV or a recombinant or derivative form thereof or its chemical equivalent.

Generally, if an HBV is used, it is first attenuated. The biological composition according to this aspect of the present invention generally further comprises one or more pharmaceutically acceptable carriers and/or diluents.

The biological composition may comprise HBsAg or like molecule from one HBV variant or the composition may be a cocktail of HbsAgs or L, M or S proteins or like molecules from a range of ADV- and/or LMV- and/or, FTC- and/or TFV-resistant HBV variants. Similar inclusions apply where the composition comprises an HBV.

The present invention is further directed to the use of defective HBV variants in the manufacture of therapeutic vaccines to vaccinate individuals against infection by HBV strains having a particular nucleotide sequence or encoding a particular polymerase or surface antigen or L, M or S proteins.

Examples of suitable vaccine candidates are defective forms of HBV variants comprising a mutation selected from, in one embodiment, in one embodiment, rtT38K, rtR55H and rtA181V; in another embodiment includes rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment includes rtN238T; and yet another embodiment includes rtI122V and rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment includes rtT128N and rtN236T, in yet another embodiment includes tL180M, rtM204V rtQ215S, in yet another

embodiment includes rtT128S rtL180M, rtM204Vand rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment includes rtN238T/A, in yet another embodiment includes rtI187V, or a combination thereof or an equivalent mutation; in a further embodiment, sQ30K. sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, or a combination thereof or an equivalent mutation.

In one embodiment, for example, an HBV variant may be identified having a particular mutation in its polymerase conferring resistance or decreased sensitivity to a nucleoside analog. This variant may then be mutated to render it defective, i.e. attenuated or unable to cause infection. Such a defective, nucleoside analog-resistant virus may then be used as a therapeutic vaccine against virulent viruses having the same mutation in its polymerase.

The subject invention extends to kits for assays for variant HBV resistant to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV. Such kits may, for example, contain the reagents from PCR or other nucleic acid hybridization technology or reagents for immunologically based detection techniques. A particularly useful assay includes the reagents and components required for immobilized oligonucleotide- or oligopeptide-mediated detection systems.

Still another aspect of the present invention contemplates a method for determining the potential for an HBV to exhibit reduced sensitivity to ADV, LMV, TFV, or FTC; or ADV

and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV and/or optionally other nucleoside or nucleotide analogs or other anti-HBV agents or combination thereof, said method comprising isolating DNA or corresponding mRNA from said HBV and screening for a mutation in the nucleotide sequence encoding HBV DNA polymerase resulting in at least one amino acid substitution, deletion and/or addition in any one or more of domains F and G, and domains A through to E or a region proximal thereto of said DNA polymerase and associated with resistance or decreased sensitivity to ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV, wherein the presence of such a mutation is an indication of the likelihood of resistance to said ADV, LMV, TFV, or FTC; or ADV and LMV; ADV and TFV; LMV and TFV; FTC and ADV; FTC and TFV; FTC and LMV; or ADV and LMV and TFV; or ADV and FTC and TFV; TFV and FTC and LMV; ADV and LMV and FTC; or ADV and FTC and LMV and TFV.

An assessment of a potential viral variant is important for selection of an appropriate therapeutic protocol. Such an assessment is suitably facilitated with the assistance of a computer programmed with software, which *inter alia* adds input codes for at least two features associated with the viral variants to provide a value corresponding to the resistance or sensitivity of a viral variant to a particular chemical compound or immunological agent. The I_Vs can be selected from (a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent; (b) an altered DNA polymerase from wild-type HBV; (c) an altered surface antigen from wild-type HBV; or (d) morbidity or recovery potential of a patient. Thus, in accordance with the present invention, I_Vs for such features are stored in a machine-readable storage medium, which is capable of processing the data to provide a value for a particular viral variant or a biological specimen comprising same.

Thus, in another aspect, the invention contemplates a computer program product for assessing the likely usefulness of a viral variant or biological sample comprising same for determining an appropriate therapeutic protocol in a subject (Figure 3), said product comprising:

(1) code that receives as input code for at least two features associated with said viral agents or biological sample comprising same, wherein said features are selected from:

(a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent;
(b) an altered DNA polymerase from wild-type HBV;
(c) an altered surface antigen from wild-type HBV; or
(d) morbidity or recovery potential of a patient;

(2) code that adds said input code to provide a sum corresponding to a value for said viral variants or biological samples; and

(3) a computer readable medium that stores the codes.

In a related aspect, the invention extends to a computer for assessing the likely usefulness of a viral variant or biological sample comprising same in a subject, wherein said computer comprises:

(1) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said machine-readable data comprise input codes for at least two features associated with said viral variant or biological sample; wherein said features are selected from:-

(a) the ability to exhibit resistance for reduced sensitivity to a particular compound or immunological agent;
(b) an altered DNA polymerase from wild-type HBV;
(c) an altered surface antigen from wild-type HBV; or
(d) morbidity or recovery potential of a patient;

(2) a working memory for storing instructions for processing said machine-readable data;

(3) a central-processing unit coupled to said working memory and to said machine-readable data storage medium, for processing said machine readable data to provide a sum of said input code corresponding to a value for said compound(s); and

(4) an output hardware coupled to said central processing unit, for receiving said value.

Any general or special purpose computer system is contemplated by the present invention and includes a processor in electrical communication with both a memory and at least one input/output device, such as a terminal. Figure 3 shows a generally suitable computer system. Such a system may include, but is not limited, to personal computers, workstations or mainframes. The processor may be a general purpose processor or microprocessor or a specialized processor executing programs located in RAM memory. The programs may be placed in RAM from a storage device, such as a disk or pre-programmed ROM memory. The RAM memory in one embodiment is used both for data storage and program execution. The computer system also embraces systems where the processor and memory reside in different physical entities but which are in electrical communication by means of a network.

In an alternative embodiment, the program screens for a mutation selected from, in one embodiment, in one embodiment, rtT38K, rtR55H and rtA181V; in another embodiment

includes rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S, rtE/K218E, rtN/H238H and rtY245H; in yet another embodiment includes rtN238T; and yet another embodiment includes rtI122V and rtA181T; in yet another embodiment includes rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P, rtV253G, in yet another embodiment includes rtT128N and rtN236T, in yet another embodiment includes tL180M, rtM204V rtQ215S, in yet another embodiment includes rtT128S rtL180M, rtM204Vand rtQ215S, in yet another embodiment includes rtI80L, rtI204M, rtN238T, in yet another embodiment includes rtN238T/A, in yet another embodiment includes rtI187V, or a combination thereof or an equivalent mutation; in a further embodiment, sQ30K. sE44G, sA47T, sI126T, sA159V and sL173F, in another embodiment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R; in yet another embodiment include sV14A, sL95W, sV96G, and sI208T/I; and in yet another embodiment include sT47A and sW172stop, and in yet another embodiment include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M, and in yet another embodiment include sS53L, and in yet another embodiment include sP120T, and in yet another embodiment include sN40S, sS207R, and in yet another embodiment include sQ101R, sI195M, sS207R, and in yet another embodiment include sL95W and sL196W, and in yet another embodiment include sV14A, or a combination thereof or an equivalent mutation.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Overlapping genome of HBV

The overlapping genome of HBV is represented in Figure 1. The gene encoding DNA polymerase (P), overlaps the viral envelope genes, Pre-S1 and Pre-S2, and partially overlaps the X and core (C) genes. The HBV envelope comprises small, middle and large proteins HBV surface antigens. The large protein component is referred to as the HBV surface antigen (HBsAg) and is encoded by the S gene sequence. The Pre-S1 and Pre-S2 gene sequences encode the other envelope components.

EXAMPLE 2

Patients on ADV Treatment and Analysis of HBV DNA

Patient A: During ADV treatment, unique HBV mutations were detected by sequencing (Table 4). This includes the unique mutation at rtT38K, and rtA181V. A number of other changes were also detected in the polymerase rtR55H and in the overlapping envelope gene (Table 4, Figures 4, 5 and 6). The changes in the HBsAg include sQ30K, sE44G, sA47T, sI126T, sA159V and sL173F. These unique changes were compared to reference sequences from each of the seven genotypes A-G as well as a consensus sequence from pretreatment samples to determine unique changes

Patient B: The HBV mutations during ADV treatment are listed in Table 5 and Figures 7, 8, and 9. The unique changes in the rt region of the HBV DNA polymerase include rtY245H. Other changes in the HBV polymerase while on ADV treatment include rtS/T78S, rtV80L, rtN/S118N, rtN/K139K, rtE142V, rtA/T181A rtI204M, rtQ/P/S/Stop215S,rt E/K218E, rtN/H238H. The changes in the HBsAg while on ADV treatment include sF55S, sC/Stop69C, sC/Y76Y, sI/V110I, sN/T131N, sN134Y, sStop/W172W, sStop/W196W, sS/R207R.

Patient C : The HBV mutations prior to ADV treatment and during ADV treatment are listed in Table 6 and Figures 10, 11 and 12. The unique changes in the rt region of the

HBV DNA polymerase while on ADV treatment include rtN238T. The unique changes in the HBsAg include sV14A, sL95W, sV96G, and sI208T/I

Patient D: The HBV mutations during ADV treatment is listed in Table 7 and Figures 13, 14 and 15. The unique changes in the HBV DNA polymerase include rtI122V and rtA181T. The unique changes in the surface include sT47A and sW172stop.

Patient E. This patient was previously treated with lamivudine and selected the unique mutations rtH237H/P while on LMV. This patient did not respond to ADV treatment Changes in the polymerase on ADV treatment include rtL180M, rtA/V200V, rtM204V, rtV214A, rtH237H/P and rtV253G. The unique changes in the surface include PreS2 T6S, sT47A, sP62L, sL/F192F, sI195M. NB: Patient on TFV and responded
Changes on ADV listed in Table 8 and Figures 16, 17 and 18.

Patient F: Unique changes during ADV treatment include the polymerase mutations at rtN238T and envelope mutations at sS53L. Changes on ADV listed in Table 9 and Figures 19, 20 and 21.

Patient G: Unique mutations while on ADV treatment include changes in the polymerase rtT128N and rtN236T and a change in envelope sP120T. Changes on ADV listed in Table 10 and Figures 22, 23 and 24.

Patient H: Mutations in the polymerase gene while on ADV treatment include rtL180M, rtM204V rtQ215S. Changes in envelope gene includesN40S, sS207R. Changes on ADV listed in Table 11 and Figures 25, 26 and 27.

Patient I: Mutations in the polymerase gene while on ADV treatment include rtT128S rtL180M, rtM204Vand rtQ215S, while mutations in the envelope gene included sQ101R, sI195M, sS207R. Changes on ADV listed in Table 12 and Figures 28, 29 and 30.

Patient J: Mutations in the polymerase gene included rtI80L, rtI204M, rtN238T and mutations in envelope sL95W and sL196W during ADV treatment. Changes on ADV listed in Table 13 and Figures 31, 32 and 33.

Patient K: Mutations in the polymerase gene at rtN238T/A was detected during Adv treatment. No changes in envelope were detected during treatment. Changes on ADV listed in Table 14 and Figures 34, 35 and 36.

Patient L: Mutations in the polymerase gene at rtI187V was detected during ADV treatment. A mutation in the envelope gene at sV14A was also detected. Changes on ADV listed in Table 15 and Figures 37, 38 and 39.

EXAMPLE 3

Detection of Viral Markers

Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), anti-HBe and hepatitis B core antigen (HBcAg) specific IgG and IgM were measured using commercially available immunoassays (Abbott Laboratories, North Chicago, IL, USA). Hepatitis B viral DNA levels were measured using a capture hybridization assay according to the manufacturer's directions (Digene Hybrid Capture II, Digene Diagnostics Inc., Beltsville, MD). The manufacturers stated cut-off for detecting HBV viremia in clinical specimens was 0.7×10^6 copies/ml or 2.5 pg/ml, [Hendricks *et al., Am J Clin Pathol 104:* 537-46, 1995]. HBV DNA levels can also be quantitated using other commercial kits such as Cobas amplification HBV monitor kit (Roche).

EXAMPLE 4

Sequencing of HBV DNA

HBV DNA was extracted from 100 µl of serum as described previously by Aye *et al., J. Hepatol. 26:* 1148-1153, 1997. Oligonucleotides were synthesized by Geneworks,

Adelaide, Australia. Amplification of the HBV polymerase gene has been described by Aye *et al.*, 1997, *supra.*

The specific amplified products were purified using PCR purification columns from MO BIO Laboratories Inc (La Jolla, CA) and directly sequenced using Big Dye terminator Cycle sequencing Ready Reaction Kit (Perkin Elmer, Cetus Norwalk, CT). The PCR primers were used as sequencing primers, **OS1** 5'- GCC TCA TTT TGT GGG TCA CCA TA-3' (nt 1408-1430) [SEQ ID NO:3], **TTA3** 5'-AAA TTC GCA GTC CCC AAA-3'(nt2128-2145) [SEQ ID NO:4], **JM** 5'-TTG GGG TGG AGC CCT CAG GCT - 3'(nt1676-1696) [SEQ ID NO:5], **TTA4** 5'-GAA AAT TGG TAA CAG CGG -3' (nt 2615-2632) [SEQ ID NO:6], OS2 5' TCT CTG ACA TAC TTT CCA AT 3' (nt 2798-2817) [SEQ ID NO:7], to sequence the internal regions of the PCR products.

EXAMPLE 5

Adefovir Dipivoxil (ADV)

ADV (formerly Bis-pom PMEA)) is a potent inhibitor of HBV replication. The structure of ADV is shown in Figure 2 and its synthesis is described by Benzaria *et al., J Med Chem. 39:* 4958-4965, 1996).

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features

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Table 4: Patient A HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV viral load	HBV RT Mutations	HBsAg Mutations
ADV	22/10/02	8.77E+06	-	sT47A sT131T/I
ADV	14/01/03	1.21 E+09	-	-
ADV	8/7/03	7.92 E+07	rtT38K rtA181V	sL173F

Table 5: Patient B RT and Polymerase mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	13/02/03	rtV80L - rtN118N/S rtN139N/K - rtV142E rtA181A/T rtI204M rtQ/P/S/Stop215Q rtE218K/E rtN238N/H	- sC76Y/C sI110V/I sN131N/T sY134N sW172Stop/W sStop196W sR/S207S
ADV	21/03/03	rtS78T/S rtV80L - rtN118N/S rtN139N/K - rtV142E rtA181A/T rtI204M rtQ215 Q/P/S/Stop rtE218K/E rtN238N/H	sS55F sC69Stop - sC76Y/C sI110V/I sN131N/T - sY134N sW172Stop/W sStop/W196W sS207S/R

ADV	22/07/03	rtS/T78S rtV80L rtN/S118N rtN/K139K - rtE142V rtA/T181A - rtI204M rtQ/P/S/Stop21 rt5SE/K218E rtN/H238H rtY245H	sF55S sC/Stop69C sC/Y76Y sI/V110I sN/T131N - sN134Y sStop/W172W sStop/W196W sS/R207R

Table 6: Patient C HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	18/08/03	rtN238T	sV14A sL95W sV96G sI208T/I

Table 7: Patient D HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	20/08/03	rtI122V rtA181T	sT47A sW172Stop

Table 8: Patient E HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
LMV	23/05/02	rtL180M rtA200V/A rtM204V rtV214A rtP237H	- sL192L/F sI195M
ADV	17/07/03	- - rtL180M rtA/V200V rtM204V rtV214A rtH237H/P rtV253G	PreS2 T6S sT47A sP62L sL/F192F sI195M

Table 9 : Patient F HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	16/10/03	rtN238T	sS53L

Table 10: Patient G HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	12/11/03	rtT128N rtN236T	sP120T

Table 11: Patient H HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	05/11/03	rtL180M rtM204V rtQ215S	sN40S sI195M sS207R

Table 12: Patient I HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	05/02/03	- rtT128S rtL180M rtM204V rtQ215S -	sQ101R - - sI195M - sS207R

Table 13: Patient J HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	18/11/03	rtI80L - rtI204M rtN238T	- sL95W sL196W -

Table 14: Patient K HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	31/03/03	rtN238T/A	No changes

Table 15: Patient L HBV Polymerase and envelope mutations detected during ADV therapy

Treatment	Date	HBV RT Mutations	HBsAg Mutations
ADV	17/09/03	rtI187V	sV14A

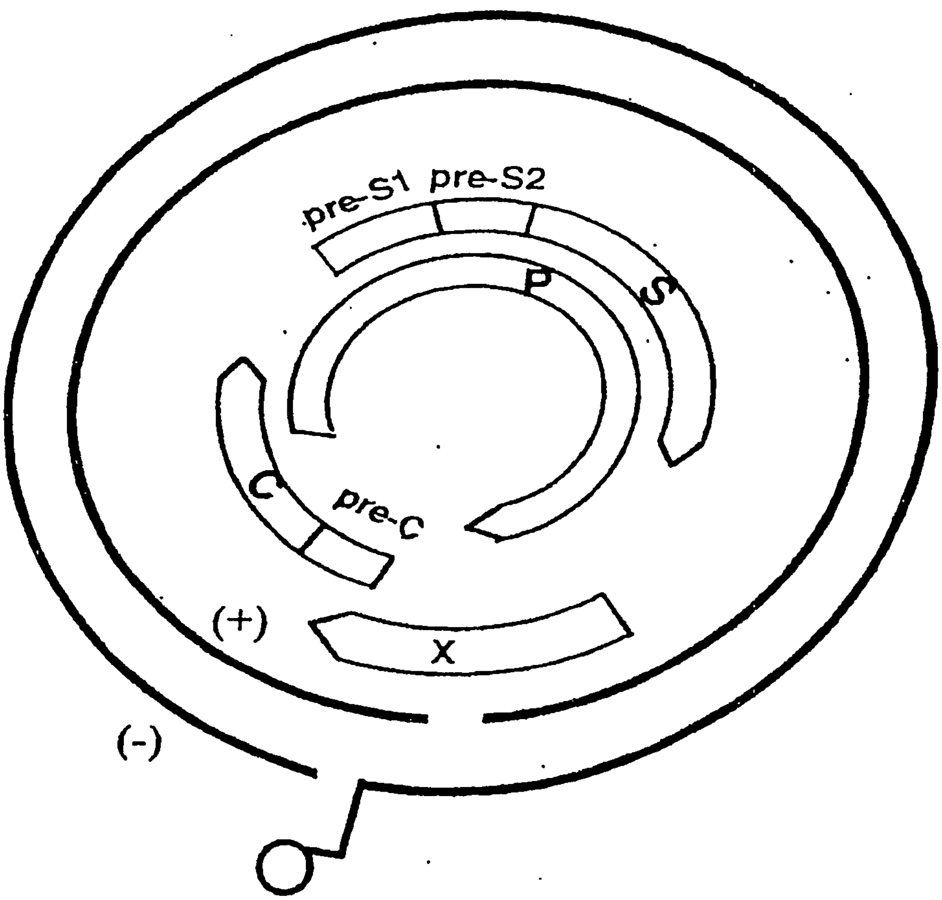


Figure 1

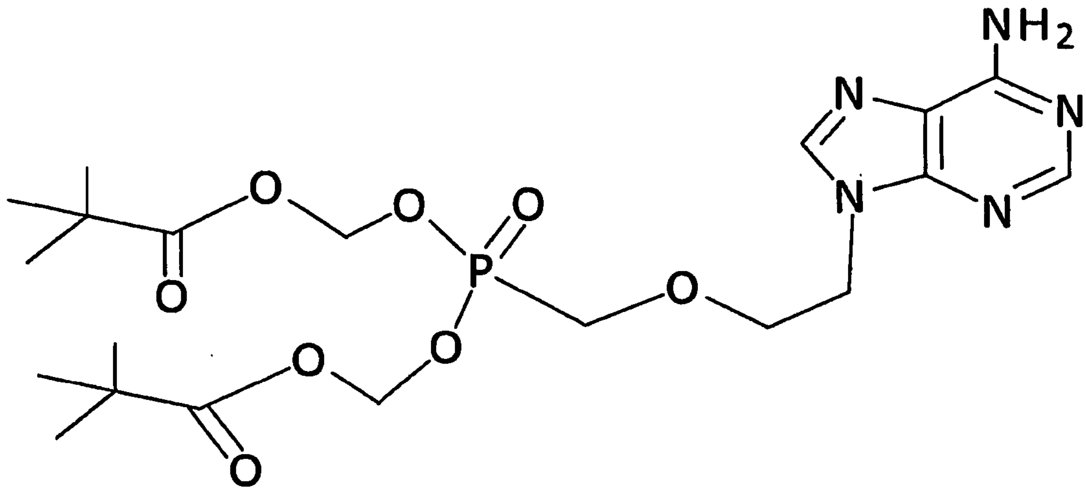
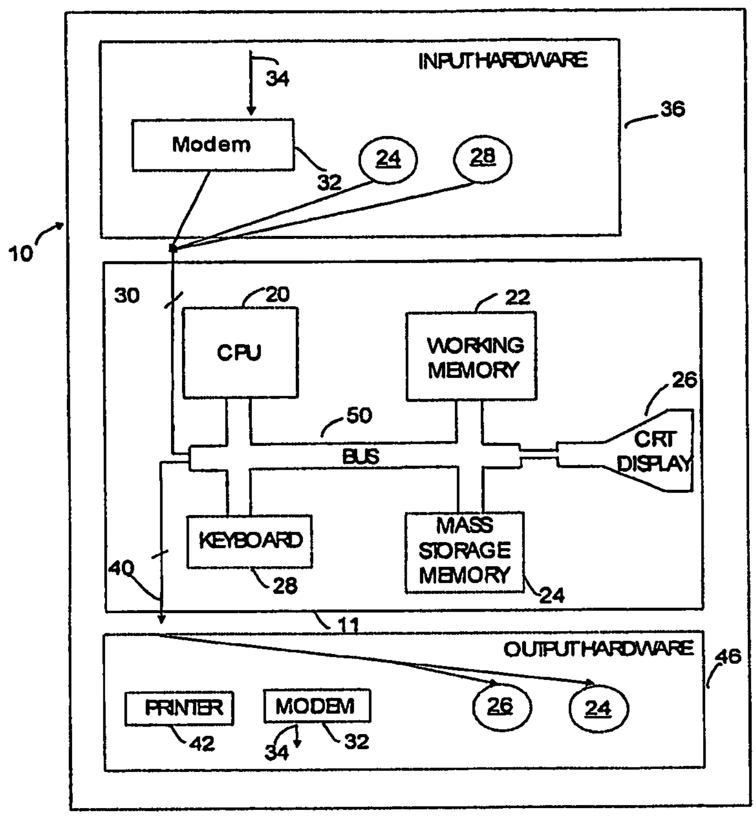


Figure 2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36
Modem
32
24
28
10
30
20
CPU
22
WORKING MEMORY
26
50
CRT DISPLAY
BUS
KEYBOARD
MASS STORAGE MEMORY
40
28
24
11
OUTPUT HARDWARE
46
PRINTER
MODEM
26
24
42
34
32

Figure 3A

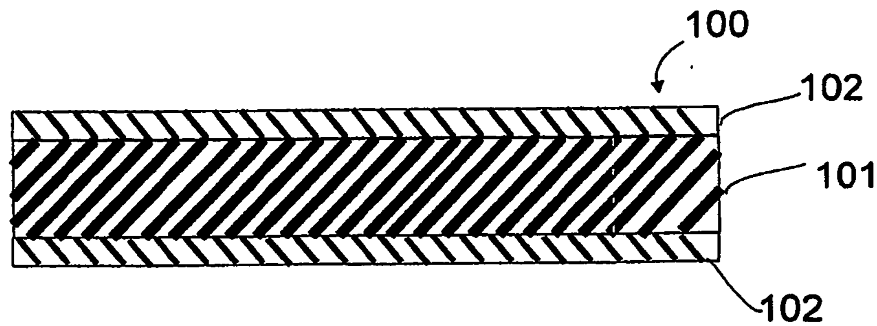

Figure 3B

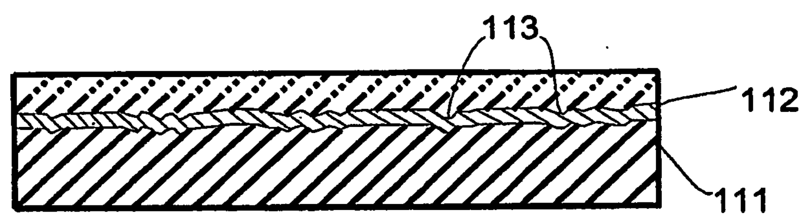

Figure 3C

Figure 4: Patient A nt sequence

```
        10        20        30        40        50
GCTTCCACCAATCGGCAGGCAGGAAGACAGCCTACTCCCATCTCTCCACC

        60        70        80        90       100
TCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCAGCACATTCC

       110       120       130       140       150
ACCATGCTCTGCTAGATCCCAGACCTGCTGGTGGCTCCAGTTCCGGAACA

       160       170       180       190       200
GTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATCTTCTCGAG

       210       220       230       240       250
GACTGGGGACCCTGCGCCGAATATGGAGAGCACCACATCAGGATTCCTAG

       260       270       280       290       300
GACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTC

       310       320       330       340       350
ACAATACCAAAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGG

       360       370       380       390       400
GGGAGCACCCACGTGTCCTGGCCAAAATTTGCAGTCCCCAACCTCCAATC

       410       420       430       440       450
ACTCACCAACCTCTTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTGT

       460       470       480       490       500
CTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTT

       510       520       530       540       550
CTTGTTGGTTCTTCTGGACTACCAAGGTATGTTGCCCGTTTGTCCTCTAC

       560       570       580       590       600
TTCCAGGAACATCAACTACCAGCACGGGACCATGCAAGACCTGCACGACT

       610       620       630       640       650
CCTGCTCAAGGAACCTCTATGTTTCCCTCTTGTTGCTGTACAAAACCTTC

       660       670       680       690       700
GGACGGAAATTGCACTTGTATTCCCATCCCATCATCTTGGGCTTTCGTAA

       710       720       730       740       750
GATTCCTATGGGAGTGGGCCTCAGTCCGTTTCTCCTGGTTCAGTTTACTA

       760       770       780       790       800
GTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTC
```

```
        810       820       830       840       850
AGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCTTGA

        860       870       880       890       900
ATCCCTTTATACCGCTATTACCAATTTTCTTTTGTCTTTGGGTATACATT

        910       920       930       940       950
TAAACCCTAATAAAACCAAGCGTTGGGGCTACTCCCTTAACTTCATGGGA

        960       970       980       990      1000
TATGTAATTGGAAGTTGGGGTACCTTGCCACAGGAACATATTGTACAAAA

AATCAAA
```

Figure 4

Figure 5: Patient A. HBV Polymerase sequence

```
          10        20        30        40        50
EDWGPCAEYGEHHIRIPRTPARVTGGVFLVDKNPHNTKESRLVVDFSQFS

          60        70        80        90       100
RGSTHVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPH

         110       120       130       140       150
LLVGSSGLPRYVARLSSTSRNINYQHGTMQDLHDSCSRNLYVSLLLLYKT

         160       170       180       190       200
FGRKLHLYSHPIILGFRKIPMGVGLSPFLLVQFTSAICSVVRRAFPHCLA

         210       220       230       240       250
FSYMDDVVLGAKSVQHLESLYTAITNFLLSLGIHLNPNKTKRWGYSLNFM

         260       270
GYVIGSWGTLPQEHIVQKIK
```

Figure 5

Figure 6: Patient A HBV HbsAg sequence

```
        10        20        30        40        50
MESTTSGFLGPLLVLQAGFFLLTRILTIPKSLDSWWTSLNFLGGAPTCPG

        60        70        80        90       100
QNLQSPTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDY

       110       120       130       140       150
QGMLPVCPLLPGTSTTSTGPCKTCTTPAQGTSMFPSCCCTKPSDGNCTCI

       160       170       180       190       200
PIPSSWAFVRFLWEWASVRFSWFSLLVPFVQWFVGLSPTVWLSVIWMMWY

       210       220
WGPSLYNILNPFIPLLPIFFCLWVYI
```

Figure 6

Figure 7: Patient B HBV NT sequence

```
        10        20        30        40        50
TCTGTCTCCACCTTTGAGAGACACTCATCCTCAGGCCATGCAGTGGAACT

        60        70        80        90       100
CCACAACCTTCCACCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTGTAT

       110       120       130       140       150
TTCCCTGCTGGTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTTC

       160       170       180       190       200
TGTCTCTCACACATCGTCAATCTTCTCGAGGATTGGGGTCCCTGCGCTGA

       210       220       230       240       250
ACATGGAGAACATCACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAG

       260       270       280       290       300
GCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGA

       310       320       330       340       350
CTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGAACTACCGTGTGTCTTG

       360       370       380       390       400
GCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCT

       410       420       430       440       450
CCAACTTGTCCTGGTTATCGCTGGATGTATCTGCGGCGTTTTATCATCTT

       460       470       480       490       500
CCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACT

       510       520       530       540       550
ATCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCTTCAACCACC

       560       570       580       590       600
AGCACGGGACCATGCAGAACCTGCACGACTCCTGCTCAAGGAAACTCTAT

       610       620       630       640       650
GTATCCCTCCTGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTA

       660       670       680       690       700
TTCCCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCC

       710       720       730       740       750
TCAGCCCGTTTCTCCTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTT
```

Figure 7

```
       760         770         780         790         800
CGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGT

       810         820         830         840         850
ATTGGGGGCCAAGTCTGTATCGCATCTTGAGTCCCTTTTTACCGCTGTTA

       860         870         880         890         900
CCAATTTTCTTTTGTCTTTGGGTATACATTTAAACCCTCACAAAACAAAA

       910         920         930         940         950
AGATGGGGTCACTCTTTACATTTCATGGGCTATGTCATTGGATGTTATGG

       960         970         980
GTCATTGCCACAAGATCACATCAGACAGAAAA
```

Figure 7 continued

Figure 8: Patient B POLYMERASE sequence

```
        10        20        30        40        50
EDWGPCAEHGEHHIRIPRTPARVTGGVFLVDKNPHNTAESRLVVDFSQFS

        60        70        80        90       100
RGNYRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPH

       110       120       130       140       150
LLVGSSGLSRYVARLSSNSRIFNHQHGTMQNLHDSCSRKLYVSLLLLYQT

       160       170       180       190       200
FGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLA

       210       220       230       240       250
FSYMDDVVLGAKSVSHLESLFTAVTNFLLSLGIHLNPHKTKRWGHSLHFM

       260
GYVIGCYGSLPQDHIRQK
```

Figure 8

Figure 9:Patient B HBsAG sequence

```
         10         20         30         40         50
MENITSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGTTVCLG

         60         70         80         90        100
QNSQSPTSNHSPTSCPPTCPGYRWMYLRRFIIFLFILLLCLIFLLVLLDY

        110        120        130        140        150
QGMLPVCPLIPGSSTTSTGPCRTCTTPAQGNSMYPSCCCTKPSDGNCTCI

        160        170        180        190        200
PIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWY

        210        220
WGPSLYRILSPFLPLLPIFFCLWVYI
```

Figure 9

Figure 10: Patient C HBV NT sequence

```
        10        20        30        40        50
CAGCAGCGCCTCCTCCTGCCTCCTCCAATCGGCAGTCAGGAAGACAGCCT

        60        70        80        90       100
ACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTG

       110       120       130       140       150
GAACTCCAGCACATTCCACCAAGCTCTGCTAGATCCCAGAGTGAGGGGCC

       160       170       180       190       200
TATATTTTCCTGCTGGTGGCTCCAGTTCCGGAACAGTAAACCCTGTTCCG

       210       220       230       240       250
ACTACTGCCTCTCCCATATCGTCAATCTTCTCGAGGACTGGGGACCCTGC

       260       270       280       290       300
ACCGAACATGGAGAGCACCACATCAGGATTCCTAGGACCCCTGCTCGCGT

       310       320       330       340       350
TACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCACAGAGT

       360       370       380       390       400
CTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGAACACCCAAGTG

       410       420       430       440       450
TCCTGGCCAAAATTTGCAGTCCCCAACCTCCAATCACTCACCAACCTCTT

       460       470       480       490       500
GTCCTCCAATTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATC

       510       520       530       540       550
ATCTTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTGGGGTCTTCT

       560       570       580       590       600
GGACTACCAAGGTATGTTGCCCGTTTGTCCTCTACTTCCAGGAACATCAA

       610       620       630       640       650
CTACCAGCACGGGACCATGCAAGACCTGCACGACTCCTGCTCAAGGAACC

       660       670       680       690       700
TCTATGTTTCCCTCTTGTTGCTGTACAAAACCTTCGGACGGAAATTGCAC
```

Figure 10

```
      710       720       730       740       750
TTGTATTCCCATCCCATCATCTTGGGCTTTCGCAAGATTCCTATGGGAGT

      760       770       780       790       800
GGGCCTCAGTCCGTTTCTCCTGGCTCAGTTTACTAGTGCCATTTGTTCAG

      810       820       830       840       850
TGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTTAGTTATATGGATGAT

      860       870       880       890       900
GTGGTATTGGGGGCCAAGTCTGTACAACAYCTTGAATCCCTTTTTACCGC

      910       920       930       940       950
TGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAAACCCTACTAAAA

      960       970       980       990      1000
CCAAACGTTGGGGCTACTCCCTTAACTTCATGGGATATGTAATTGGAAGT

     1010      1020      1030      1040
TGGGGTACCTTACCACAAGAACATATTGTACACAAAATCAGACAA
```

Figure 10 continued

Figure 11: Patient C Polymerase sequence

```
         10         20         30         40         50
EDWGPCTEHGEHHIRIPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFS

         60         70         80         90        100
RGNTQVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPH

        110        120        130        140        150
LLVGSSGLPRYVARLSSTSRNINYQHGTMQDLHDSCSRNLYVSLLLLYKT

        160        170        180        190        200
FGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLA

        210        220        230        240        250
FSYMDDVVLGAKSVQHLESLFTAVTNFLLSLGIHLNPTKTKRWGYSLNFM

        260        270
GYVIGSWGTLPQEHIVHKIRQ
```

Figure 11

Figure 12 Patient C HbsAg sequence

```
        10        20        30        40        50
MESTTSGFLGPLLALQAGFFLLTRILTIPQSLDSWWTSLNFLGGTPKCPG

        60        70        80        90       100
QNLQSPTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLWGLLDY

       110       120       130       140       150
QGMLPVCPLLPGTSTTSTGPCKTCTTPAQGTSMFPSCCCTKPSDGNCTCI

       160       170       180       190       200
PIPSSWAFARFLWEWASVRFSWLSLLVPFVQWFVGLSPTVWLLVIWMMWY

       210       220
WGPSLYNXLNPFLPLLPIFFCLWVYI
```

Figure 12

Figure 13; Patient D NT sequence

```
         10        20        30        40        50
CTCCTGCATCTACCAATCGGCAGTCAGGAAGACAGCCTACTCCCATCTCT

         60        70        80        90       100
CCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCACAAC

        110       120       130       140       150
TTTCCACCAAGCTCTGCTAGATCCCCGAGTGAGGGGCCTCTATTTTCCTG

        160       170       180       190       200
CTGGTGGCTCCAGTTCCGGGACAGTAAACCCTGTTCCGACTACTGCCTCT

        210       220       230       240       250
CCCATATCGTCAATCTTCTCGAGGACTGGGGACCCTGCACTGAACATGGA

        260       270       280       290       300
GAGCACAACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAGGCGGTGT

        310       320       330       340       350
TTTTCTTGTTGACAAGAATCCTCACAATACCACAGAGTCTAGACTCGTGG

        360       370       380       390       400
TGGACTTCTCTCAATTTTCTAGGGGAAGCACCCGCGTGTCCTGGCCAAAA

        410       420       430       440       450
TTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTCCTCCAATTT

        460       470       480       490       500
GTCCTGGCTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTC

        510       520       530       540       550
ATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGATTACCAAGG

        560       570       580       590       600
TATGTTGCCCGTTTGTCCTCTACTTCCAGGAACGTCAACTACCAGCACGG

        610       620       630       640       650
GACCATGCAAGACCTGCACGATTCCTGCTCAAGGAACCTCTATGTTTCCC

        660       670       680       690       700
TCATGTTGCTGTACAAAACCTTCGGACGGAAACTGCACTTGTATTCCCAT

        710       720       730       740       750
CCCATCATCCTGGGCTTTCGCAAGATTCCTATGGGAGTGGGCCTCAGTCC
```

Figure 13

```
       760       770       780       790       800
GTTTCTCTTGACTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGG

       810       820       830       840       850
CTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGG

       860       870       880       890       900
GCCAAGTCTGTACAACATCTTGAGTCCCTTTATACCGCTATTACCAATTT

       910       920       930       940       950
TCTTTTGTCTTTGGGTATACATTTAAACCCTAATAAAACCAAGCGATGGG

       960       970       980       990      1000
GTTACTCCCTTAACTTCATGGGATATGTCATTGGAAGTTGGGGGACTTTA

      1010      1020
CCACAGGAACATATTGTGCTC
```

Figure 13 continued

Figure 14: patient D HBV POL sequence

```
         10         20         30         40         50
EDWGPCTEHGEHNIRIPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFS

         60         70         80         90        100
RGSTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPH

        110        120        130        140        150
LLVGSSGLPRYVARLSSTSRNVNYQHGTMQDLHDSCSRNLYVSLMLLYKT

        160        170        180        190        200
FGRKLHLYSHPIILGFRKIPMGVGLSPFLLTQFTSAICSVVRRAFPHCLA

        210        220        230        240        250
FSYMDDVVLGAKSVQHLESLYTAITNFLLSLGIHLNPNKTKRWGYSLNFM

        260
GYVIGSWGTLPQEHIVL
```

Figure 14

Figure 15 Patient D HBsAg sequence

```
        10         20         30         40         50
MESTTSGFLGPLLVLQAVFFLLTRILTIPQSLDSWWTSLNFLGEAPACPG

        60         70         80         90        100
QNSQSPTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDY

       110        120        130        140        150
QGMLPVCPLLPGTSTTSTGPCKTCTIPAQGTSMFPSCCCTKPSDGNCTCI

       160        170        180        190        200
PIPSSWAFARFLWEWASVRFS*LSLLVPFVQWFVGLSPTVWLSVIWMMWY

       210        220
WGPSLYNILSPFIPLLPIFFCLWVYI
```

Figure 15

Figure 16: Patient E HBV nt sequence

```
        10         20         30         40         50
AGTCATCCTCAGGCCATGCAGTGGAACTCCAGCACATTCCACCAAGCTCT

        60         70         80         90        100
GCTAGATCCCAGAGTGAGGGGCCTATACTTTCCTGCTGGTGGCTCCAGTT

       110        120        130        140        150
CAGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCAATC

       160        170        180        190        200
TTCTCGAGGACTGGGGACCCTGCACCGAATATGGAGAGCACCACATCAGG

       210        220        230        240        250
ATTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAA

       260        270        280        290        300
GAATCCTCACAATACCACAGAGTCTAGACTCGTGGTGGACTTCTCTCAAT

       310        320        330        340        350
TTTCTAGGGGGAGCACCCGCGTGTCCTGGCCAAAATTTGCAGTCCCCAAC

       360        370        380        390        400
CTCCAATCACTCACTAACCTCTTGTCCTCCAATTTGTCCTGGTTATCGCT

       410        420        430        440        450
GGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTATGC

       460        470        480        490        500
CTCATCTTCTTGTTGGTTCTTCTGGACTACCAAGGTATGTTGCCCGTTTG

       510        520        530        540        550
TCCTCTACTTCCAGGAACATCAACTACCAGCACGGGACCATGCAAGACCT

       560        570        580        590        600
GCACGACTCCTGCTCAAGGAACCTCTATGTTTCCCTCTTGTTGTTGTACA

       610        620        630        640        650
AAACCTTCGGACGGAAATTGCACTTGTATTCCCATCCCATCATCTTGGGC

       660        670        680        690        700
TTTCGCAAGATTCCTATGGGAGTGGGCCTCAGTCCGTTTCTCATGGCTCA
```

Figure 16

```
        710        720        730        740        750
GTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTT

        760        770        780        790        800
TGGTTTTCAGTTATGTGGATGATGTGGTATTGGGGGCCAAGTCTGCACAA

        810        820        830        840        850
CATCTTGAATCCCTTTTTACCGCTATTACCAATTTTCTTTTGTCTTTGGG

        860        870        880        890        900
TATACATTTAAACCMTAATAAAACCAAACGTTGGGGCTATTCCCTTAACT

        910        920        930        940        950
TTATGGGATATGGAATTGGAAGTTGGGGTCCTGCCCAGGGAAGATGGCAG

GGG
```

Figure 16 continued

Figure 17 Patient E: HBV polymerase

```
        10         20         30         40         50
SSSGHAVELQHIPPSSARSQSEGPILSCWWLQFRNSKPCSDYCLSHIVNL

        60         70         80         90        100
LEDWGPCTEYGEHHIRIPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQF

       110        120        130        140        150
SRGSTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMP

       160        170        180        190        200
HLLVGSSGLPRYVARLSSTSRNINYQHGTMQDLHDSCSRNLYVSLLLLYK

       210        220        230        240        250
TFGRKLHLYSHPIILGFRKIPMGVGLSPFLMAQFTSAICSVVRRAFPHCL

       260        270        280        290        300
VFSYVDDVVLGAKSAQHLESLFTAITNFLLSLGIHLNXNKTKRWGYSLNF

MGYGIGSWG
```

Figure 17

Figure 18: Patient E HBsAg

```
         10        20        30        40        50
QPTPISPPLRDSHPQAMQWNSSTFHQALLDPRVRGLYFPAGGSSSGTVNP

         60        70        80        90       100
VPTTASPISSIFSRTGDPAPNMESTTSGFLGPLLVLQAGFFLLTRILTIP

        110       120       130       140       150
QSLDSWWTSLNFLGGAPACPGQNLQSPTSNHSLTSCPPICPGYRWMCLRR

        160       170       180       190       200
FIIFLFILLLCLIFLLVLLDYQGMLPVCPLLPGTSTTSTGPCKTCTTPAQ

        210       220       230       240       250
GTSMFPSCCCTKPSDGNCTCIPIPSSWAFARFLWEWASVRFSWLSLLVPF

        260       270       280       290       300
VQWFVGLSPTVWFSVMWMMWYWGPSLHNILNPFLPLLPIFFCLWVYI*TX

IKPNVGA
```

Figure 18

Figure 19: Patient F: nt sequence

```
        10         20         30         40         50
CCAATCGGCAGTCAGGAAGACAGCCTACTCCCATCTCTCCACCTCTAAGA

        60         70         80         90        100
GACAGTCATCCTCAGGCCATGCAGTGGAACTCCAGCACATTCCACCAAGC

       110        120        130        140        150
TCTGCTAGATCCCAGAGTGAGGGGCCTATACTTTCCTGCTGGTGGCTCCA

       160        170        180        190        200
GTTCCGGAACAGTAAACCCTGTTCCGACTACTGCCTCTCCCATATCGTCA

       210        220        230        240        250
ATCTTCTCGAGGACTGGGGACCCTGCACCGAATATGGAGAGCACCACATC

       260        270        280        290        300
AGGATTCCTAGGACCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGA

       310        320        330        340        350
CAAGAATCCTCACAATACCACAGAGTCTAGACTCGTGGTGGACTTCTCTC

       360        370        380        390        400
AATTTTCTAGGGGGAGCACCCACGTGTCCTGGCCAAAATTTGCAGTCCCC

       410        420        430        440        450
AACCTCCAATCACTCACCAACCTCTTGTCCTCCAATTTGTCCTGGTTATC

       460        470        480        490        500
GCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTCTTCATCCTGCTGCTA

       510        520        530        540        550
TGCCTCATCTTCTTGTTGGTTCTTCTGGACTACCAAGGTATGTTGCCCGT

       560        570        580        590        600
TTGTCCTCTACTTCCAGGAACATCAACTACCAGCACGGGACCATGCAAGA

       610        620        630        640        650
CCTGCACGACTCCTGCTCAAGGAACCTCTATGTTTCCCTCTTGTTGCTGT
```

Figure 19

```
        660       670       680       690       700
ACAAAACCTTCGGACGGAAATTGCACTTGTATTCCCATCCCATCATCTTG

        710       720       730       740       750
GGCTTTCGCAAGATTCCTATGGGAGTGGGCCTCAGTCCGTTTCTCCTGGC

        760       770       780       790       800
TCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACT

        810       820       830       840       850
GTTTGGCTTTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTA

        860       870       880       890       900
CAACATCTTGAATCCCTTTTTACCGCTGTTACCAATTTTCTTTTGTCTTT

        910       920       930       940       950
GGGTATACATTTAAACCCTACTAAAACTAAACGTTGGGGCTACTCCCTTA

        960       970       980
ACTTCATGGGATATGTAATTGGAAGTTGGGGTACCTTG
```

Figure 19 continued

Figure 20 Patient F Pol Amino acid sequence

```
        10         20         30         40         50
EDWGPCTEYGEHHIRIPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFS

        60         70         80         90        100
RGSTHVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPH

       110        120        130        140        150
LLVGSSGLPRYVARLSSTSRNINYQHGTMQDLHDSCSRNLYVSLLLLYKT

       160        170        180        190        200
FGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLA

       210        220        230        240        250
FSYMDDVVLGAKSVQHLESLFTAVTNFLLSLGIHLNPTKTKRWGYSLNFM

GYVIGSWG
```

Figure 20

Figure 21 Patient F HBsAg seq

```
        10        20        30        40        50
MESTTSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGAPTCPG

        60        70        80        90       100
QNLQSPTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLLVLLDY

       110       120       130       140       150
QGMLPVCPLLPGTSTTSTGPCKTCTTPAQGTSMFPSCCCTKPSDGNCTCI

       160       170       180       190       200
PIPSSWAFARFLWEWASVRFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWY

       210       220
WGPSLYNILNPFLPLLPIFFCLWVYI
```

Figure 21

Figure 22: Patient G ;HBV nt

```
        10        20        30        40        50
TCCGCCTCCTGCCTCCACCAATCGCCAGTCAGGAAGGCAACCTACCCCGC

        60        70        80        90       100
TCTCTCCACCTTTGAGAGACACTCATCCTCAGGCCGTGCAGTGGAACTCC

       110       120       130       140       150
ACAACCTTCCACCAAACTCTGCAAGATCCCAGAGTGAGGGGCCTGTATCT

       160       170       180       190       200
CCCTGCTGGTGGCTCCAGTTCAGGAACAGCAAACCCTGTTCCGACTACTG

       210       220       230       240       250
CCTCTCGCTTATCGTCAATCTTCTCGAGGATTGGGGACCCTGCGCTGAAC

       260       270       280       290       300
ATGGAGAACATCACATCAGGACTCCTAGGACCCCTTCTCGTGTTACAGGC

       310       320       330       340       350
GGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACT

       360       370       380       390       400
CGTGGTGGACTTCTCTCAGTTTTCTAGGGGGAACTACCGTGTGTCTTGGC

       410       420       430       440       450
CAAAATTCGCGGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCC

       460       470       480       490       500
GACTTGTCCTGGTTATCGCTGGATGTATCTGCGGCGTTTTATCATATTCC

       510       520       530       540       550
TCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTAT

       560       570       580       590       600
CAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCTCAACCACCAG

       610       620       630       640       650
CACGGGAACATGCCGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGT

       660       670       680       690       700
ATCCCTCCTGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTATT
```

Figure 22

```
       710        720        730        740        750
CCCATCCCATCATCTTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCTC

       760        770        780        790        800
AGCCCGTTTCTCCTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCG

       810        820        830        840        850
TAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTAT

       860        870        880        890        900
TGGGGGCCAAGTCTGTACAGCATCTTGAGTCCCTTTTTACCGCTGTTACC

       910        920        930        940        950
AATTTTCTTTTGTCTTTGGGTATACATTTAACCCCTAACAAAACAAAGAG

       960        970        980        990       1000
ATGGGGTTACTCTCTAAATTTTATGGGCTATGTCATTGGAAGTTATGGGT

      1010       1020       1030       1040
CCTTGCCACAAGAACACATTATACTAAAAATCAAAGATTGTTT
```

Figure 22 continued

Figure 23 Patient G HBV POL

```
         10         20         30         40         50
EDWGPCAEHGEHHIRTPRTPSRVTGGVFLVDKNPHNTAESRLVVDFSQFS

         60         70         80         90        100
RGNYRVSWPKFAVPNLQSLTNLLSSDLSWLSLDVSAAFYHIPLHPAAMPH

        110        120        130        140        150
LLVGSSGLSRYVARLSSNSRILNHQHGNMPNLHDSCSRNLYVSLLLLYQT

        160        170        180        190        200
FGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLA

        210        220        230        240        250
FSYMDDVVLGAKSVQHLESLFTAVTNFLLSLGIHLTPNKTKRWGYSLNFM

GYVIGSYG
```

Figure 23

Figure 24: Patient G HbsAg

```
        10        20        30        40        50
MENITSGLLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLSFLGGTTVCLG

        60        70        80        90       100
QNSRSPTSNHSPTSCPPTCPGYRWMYLRRFIIFLFILLLCLIFLLVLLDY

       110       120       130       140       150
QGMLPVCPLIPGSSTTSTGTCRTCTTPAQGTSMYPSCCCTKPSDGNCTCI

       160       170       180       190       200
PIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWY

       210       220
WGPSLYSILSPFLPLLPIFFCLWVYI
```

Figure 24

Figure 25 Patient H nt seq

```
        10        20        30        40        50
CGCCTCCTGCCTCCACCAATCGCCAGTCAGGAAGGCAGCCGACCCCACTG

        60        70        80        90       100
TCTCCACCTTTGAGAGACACTCATCCTCAGGCCGTGCAGTGGAACTCCAC

       110       120       130       140       150
AACCTTCCACCAAACTCTGCAAGATCCCAGAGTGAGAGGCCTGTATTTCC

       160       170       180       190       200
CTGCTGGTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACCACTGCC

       210       220       230       240       250
TCTCCCTTATCGTCAATCTTCTCGAGGATTGGGGACCCTGCGCTGAACAT

       260       270       280       290       300
GGAGAACATCACATCAGGATTCCTAGGACCCCTTCTCGTGTTACAGGCGG

       310       320       330       340       350
GGTTTTTCTTGTTGACAAGAATCCTCACAATACCGCAGAGTCTAGACTCG

       360       370       380       390       400
TGGTGGACTTCTCTCAGTTTTCTAGGGGAAACCACCGTGTGTCTTGGCCA

       410       420       430       440       450
AAATTCGCAGTCCCCAACCTCCAATCACTCACCAACCTCCTGTCCTCCAA

       460       470       480       490       500
CTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATATTCCTC

       510       520       530       540       550
TTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTATCA

       560       570       580       590       600
AGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGATCCTCAACCACCAGCA

       610       620       630       640       650
CGGGACCATGCCGAACCTGCACGACTCCTGCTCAAGGAACCTCTATGTAT

       660       670       680       690       700
CCCTCCTGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTATTCC
```

Figure 25

```
        710       720       730       740       750
CATCCCATCATCTTGGGCTTTCGCAAAATTCCTATGGGAGTGGGGCTCAG

        760       770       780       790       800
CCCGTTTCTCATGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTA

        810       820       830       840       850
GGGCTTTCCCCCACTGTTTGGCTTTCAGTTATGTGGATGATGTGGTATTG

        860       870       880       890       900
GGGGCCAAGTCTGTATCGCATCTTGAGTCCCTTTTTACCGCTGTTACCAA

        910       920       930       940       950
TTTTCTTTTGTCTTTGGGTATACATTTAAACCCTAACAAAACGAAAAGAT

        960       970       980       990      1000
GGGGTTACTCTTTAAATTTTATGGGGTATGTTATTGGATGTTATGGGTCC

       1010      1020
TTGCCACAAGAACACATCGTACAAAAA
```

Figure 25 continued

Figure 26: Patient H HBV pol

```
        10        20        30        40        50
EDWGPCAEHGEHHIRIPRTPSRVTGGVFLVDKNPHNTAESRLVVDFSQFS

        60        70        80        90       100
RGNHRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHIPLHPAAMPH

       110       120       130       140       150
LLVGSSGLSRYVARLSSNSRILNHQHGTMPNLHDSCSRNLYVSLLLLYQT

       160       170       180       190       200
FGRKLHLYSHPIILGFRKIPMGVGLSPFLMAQFTSAICSVVRRAFPHCLA

       210       220       230       240       250
FSYVDDVVLGAKSVSHLESLFTAVTNFLLSLGIHLNPNKTKRWGYSLNFM

       260
GYVIGCYGSLPQEH
```

Figure 26

Figure 27: Patient H HBsAg

```
         10         20         30         40         50
MENITSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLSFLGETTVCLG

         60         70         80         90        100
QNSQSPTSNHSPTSCPPTCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDY

        110        120        130        140        150
QGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSMYPSCCCTKPSDGNCTCI

        160        170        180        190        200
PIPSSWAFAKFLWEWGSARFSWLSLLVPFVQWFVGLSPTVWLSVMWMMWY

        210        220
WGPSLYRILSPFLPLLPIFFCLWVYI
```

Figure 27

Figure 28 Patient I HBV nt seq

```
         10         20         30         40         50
CAACTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATATTC

         60         70         80         90        100
CTCTTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTA

        110        120        130        140        150
TCGAGGTATGTTGCCCGTTTGTCCTCTACTTCCAGGATCTTCAACCACCA

        160        170        180        190        200
GCACGGGTCCATGCAGAACCTGCACGACTCCTGCTCAAGGAACCTCTATG

        210        220        230        240        250
TATCCCTCATGTTGCTGTACCAAACCTTCGGACGGAAATTGCACCTGTAT

        260        270        280        290        300
TCCCATCCCATCATCCTGGGCTTTCGGAAAATTCCTATGGGAGTGGGCCT

        310        320        330        340        350
CAGCCCGTTTCTCATGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTC

        360        370        380        390        400
GTAGGGCTTTCCCCCATTGTTTGGCTTTCAGTTATGTGGATGATGTGGTA

        410        420        430        440        450
TTGGGGGCCAAGTCTGTATCGCATCTTGAGTCCCTTTTTACCGCTGTTAC

        460        470        480        490        500
CAATTTTCTTTTGTCTCTGGGTATACATTTAAACCCTCACAAAACAAAAA

        510        520        530        540        550
GATGGGGTTACTCTTTACATTTCATGGGCTATGTCATCGGATGTTATGGG

        560
TCTTTGCCAC
```

Figure 28

Figure 29 Patient I HBV pol

```
         10         20         30         40         50
NLSWLSLDVSAAFYHIPLHPAAMPHLLVGSSGLSRYVARLSSTSRIFNHQ

         60         70         80         90        100
HGSMQNLHDSCSRNLYVSLMLLYQTFGRKLHLYSHPIILGFRKIPMGVGL

        110        120        130        140        150
SPFLMAQFTSAICSVVRRAFPHCLAFSYVDDVVLGAKSVSHLESLFTAVT

        160        170        180
NFLLSLGIHLNPHKTKRWGYSLHFMGYVIGCYGSLP
```

Figure 29

Figure 30 Patient I: HBsAg

```
        10         20         30         40         50
TCPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYRGMLPVCPLLPGSSTTS

        60         70         80         90        100
TGPCRTCTTPAQGTSMYPSCCCTKPSDGNCTCIPIPSSWAFGKFLWEWAS

       110        120        130        140        150
ARFSWLSLLVPFVQWFVGLSPIVWLSVMWMMWYWGPSLYRILSPFLPLLP

       160        170        180
IFFCLWVYI*
```

Figure 30

Figure 31 Patient J HBV nt seq

```
        10        20        30        40        50
CGCCTCCTCCTGCCTCCACCATCGGCAGTCAGGAAGAAAGCCTACTCCCA

        60        70        80        90       100
TCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCC

       110       120       130       140       150
AGCACATTCCACCAAGCTCTGCTAGATCCCARAGTGAGRGGCCTATACTT

       160       170       180       190       200
TCCTGCTGGTGGCTCCAGTTCCGGAACAGTAAACCCTGTTCCGACTACTG

       210       220       230       240       250
CCTCTCCCATATCGTCAATCTTCTCGAGGACTGGGGACCCTGCACCGAAT

       260       270       280       290       300
ATGGAGAGCACAACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAGGC

       310       320       330       340       350
GGGGTTTTTCTTGTTGACAAGAATCCTCACAATACCACAGAGTCTAGACT

       360       370       380       390       400
CGTGGTGGACTTCTCTCAATTTTCTAGGGGGAGCACCCACGTGTCCTGGC

       410       420       430       440       450
CAAAATTTGCAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTCCTCC

       460       470       480       490       500
AATTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCC

       510       520       530       540       550
TCTTCATCCTGCTGCTATGCCTCATCTTCTTGTKGGTTCTTCTGGACTAC

       560       570       580       590       600
CAAGGTATGTTGCCCGTTTGTCCTCTACTTCCAGGAACATCAACTACCAG

       610       620       630       640       650
CACGGGACCATGCAAGACCTGCACGATTCCTGCTCAAGGAACCTCTATGT

       660       670       680       690       700
TTCCCTCTTGTTGCTGTACAAAACCTTCGGACGGAAATTGCACTTGTATT
```

Figure 31

```
        710         720         730         740         750
CCCATCCCATCATCTTGGGCTTTCGCAAGATTCCTATGGGAGTGGGCCTC

        760         770         780         790         800
AGTCCGTTTCTCCTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCG

        810         820         830         840         850
TAGGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTAT

        860         870         880         890         900
TGGGGGCCAAGTCTGTACAACATCTTGAATCCCTTTTTACCGCTGTTACC

        910         920         930         940         950
AATTTTCTTTTGTCTTTGGGTATACATTTAAACCCTACTAAAACTAAACG

        960         970         980         990        1000
TTGGGGCTACTCCCTTAACTTCATGGGATATGTAATTGGAAGTTGGGGTA

       1010        1020
CCTTACCACAGGAACATATTGTACACAAA
```

Figure 31 continued

Figure 32 Patient J HBV pol

```
         10         20         30         40         50
EDWGPCTEYGEHNIRIPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFS

         60         70         80         90        100
RGSTHVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPH

        110        120        130        140        150
LLVGSSGLPRYVARLSSTSRNINYQHGTMQDLHDSCSRNLYVSLLLLYKT

        160        170        180        190        200
FGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLA

        210        220        230        240        250
FSYMDDVVLGAKSVQHLESLFTAVTNFLLSLGIHLNPTKTKRWGYSLNFM

        260
GYVIGSWGTLPQEHIVHK
```

Figure 32

Figure 33. Patient J HBsAg

```
         10        20        30        40        50
MESTTSGFLGPLLVLQAGFFLLTRILTIPQSLDSWWTSLNFLGGAPTCPG

         60        70        80        90       100
QNLQSPTSNHSPTSCPPICPGYRWMCLRRFIIFLFILLLCLIFLXVLLDY

        110       120       130       140       150
QGMLPVCPLLPGTSTTSTGPCKTCTIPAQGTSMFPSCCCTKPSDGNCTCI

        160       170       180       190       200
PIPSSWAFARFLWEWASVRFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWY

        210       220
WGPSLYNILNPFLPLLPIFFCLWVYI
```

Figure 33

Figure 34 Patient K HBV nt

```
         10        20        30        40        50
CTCCTCCTGCCTCCACCAATCGGCAGTCAGGAAGACAGCCTACACCCATC

         60        70        80        90       100
TCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATGCAGTGGAACTCCAG

        110       120       130       140       150
CACATTCCACCAAGCTCTGCTAGATCCCAGAGTGAGGGGCCTATACTTTC

        160       170       180       190       200
CTGCTGGTGGCTCCAGTTCAGGAACAGTAAACCCTGTTCCGACTACTGCC

        210       220       230       240       250
TCTCCCATATCGTCAATCTTCTCGAGGACTGGGGACCCTGCACCGAATAT

        260       270       280       290       300
GGAGAGCACCACATCAGGATTCCTAGGACCCCTGCTCGTGTTACAGGCGG

        310       320       330       340       350
GGTTTTTCTTGTTGACAAGAATCCTCACAATACCACAGAGTCTAGACTCG

        360       370       380       390       400
TGGTGGACTTCTCTCAATTTTCTAGGGGGAGCACCCACGTGTCCTGGCCA

        410       420       430       440       450
AAATTTGCAGTCCCCAACCTCCAATCACTCACCAACCTCTTGTCCTCCAA

        460       470       480       490       500
TTTGTCCTGGTTATCGCTGGATGTGTCTGCGGCGTTTTATCATCTTCCTC

        510       520       530       540       550
TTCATCCTGCTGCTATGCCTCATCTTCTTGTTGGTTCTTCTGGACTACCA

        560       570       580       590       600
AGGTATGTTGCCCGTTTGTCCTCTACTTCCAGGAACATCAACTACCAGCA

        610       620       630       640       650
CGGGACCATGCAAGACCTGCACGATTCCTGCTCAAGGAACCTCTATGTTT

        660       670       680       690       700
CCCTCTTGTTGCTGTACAAAACCTTCGGACGGAAATTGCACTTGTATTCC
```

Figure 34

```
        710         720         730         740         750
CATCCCATCATCTTGGGCTTTCGCAAGATTCCTATGGGAGTGGGCCTCAG

        760         770         780         790         800
TCCGTTTCTCCTGGCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTA

        810         820         830         840         850
GGGCTTTCCCCCACTGTTTGGCTTTCAGTTATATGGATGATGTGGTATTG

        860         870         880         890         900
GGGGCCAAGTCTGTACAACATCTTGAATCCCTTTTTACCGCTGTTACCAA

        910         920         930         940         950
TTTTCTTTTGTCTTTGGGTATACATTTAAACCCTRCTAAAACCAAACGTT

        960         970         980         990        1000
GGGGTTACTCCCTTAACTTCATGGGATATGTAATTGGAAGTTGGGGTACC

       1010        1020        1030
TTACCACAGGAACATATTGTACACAAAATCAAACA
```

Figure 34 continued

Figure 35 Patient K HBV pol

```
         10        20        30        40        50
SSCLHQSAVRKTAYTHLSTSKRQSSSGHAVELQHIPPSSARSQSEGPILS

         60        70        80        90       100
CWWLQFRNSKPCSDYCLSHIVNLLEDWGPCTEYGEHHIRIPRTPARVTGG

        110       120       130       140       150
VFLVDKNPHNTTESRLVVDFSQFSRGSTHVSWPKFAVPNLQSLTNLLSSN

        160       170       180       190       200
LSWLSLDVSAAFYHLPLHPAAMPHLLVGSSGLPRYVARLSSTSRNINYQH

        210       220       230       240       250
GTMQDLHDSCSRNLYVSLLLLYKTFGRKLHLYSHPIILGFRKIPMGVGLS

        260       270       280       290       300
PFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKSVQHLESLFTAVTN

        310       320       330       340
FLLSLGIHLNPXKTKRWGYSLNFMGYVIGSWGTLPQEHIVHKIK
```

Figure 35

Figure 36 Patient K HbsAg

```
        10         20         30         40         50
PPASTNRQSGRQPTPISPPLRDSHPQAMQWNSSTFHQALLDPRVRGLYFP

        60         70         80         90        100
AGGSSSGTVNPVPTTASPISSIFSRTGDPAPNMESTTSGFLGPLLVLQAG

       110        120        130        140        150
FFLLTRILTIPQSLDSWWTSLNFLGGAPTCPGQNLQSPTSNHSPTSCPPI

       160        170        180        190        200
CPGYRWMCLRRFIIFLFILLLCLIFLLVLLDYQGMLPVCPLLPGTSTTST

       210        220        230        240        250
GPCKTCTIPAQGTSMFPSCCCTKPSDGNCTCIPIPSSWAFARFLWEWASV

       260        270        280        290        300
RFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWYWGPSLYNILNPFLPLLPI

       310        320        330        340
FFCLWVYI*TLLKPNVGVTPLTSWDM*LEVGVPYHRNILYTKSN
```

Figure 36

Figure 37 Patient L HBV nt

```
        10         20         30         40         50
CAGTCCGGAAGGCAGCCTACTCCCTTATCTCCACCTCTAAGGGACACTCA

        60         70         80         90        100
TCCTCAGGCCATGCAGTGGAACTCCACCACTTTCCATCAAACTCTTCAAG

       110        120        130        140        150
ATCCCAGAGTCAGGGCTCTGTACTTTCCTGCTGGTGGCTCCAGTTCAGGA

       160        170        180        190        200
ACAGTGAGCCCTGCTCAGAATACTGCCTCTGCCATATCGTCAACCTTCTC

       210        220        230        240        250
GAAGACTGGGGACCCTGTACCGAACATGGAGAACATCGCATCAGGACTCC

       260        270        280        290        300
TAGGACCCCTGCTCGCGTTACAGGCGGGGTTTTTCTCGTTGACAAAAATC

       310        320        330        340        350
CTCACAATACCACAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCT

       360        370        380        390        400
AGGGGGAACACCCGTGTGTCTTGGCCAAAATTCGCAGTCCCAAATCTCCA

       410        420        430        440        450
GTCACTCACCAACTTGTTGTCCTCCAATTTGTCCTGGTTATCGCTGGATG

       460        470        480        490        500
TGTCTGCGGCGTTTTATCATCTTCCTCTGCATCCTGCTGCTATGCCTCAT

       510        520        530        540        550
CTTCTTGTTGGTTCTTCTGGACTATCAAGGTATGTTGCCCGTTTGTCCTC

       560        570        580        590        600
TAATTCCAGGATCATCAACCACCAGCACCGGACCATGCAGAACCTGCACG

       610        620        630        640        650
ACTCCTGCTCAAGGAACCTCTATGTTTCCCTCATGTTGCTGTACAAAACC
```

Figure 37

```
       660        670        680        690        700
TACGGACGGAAACTGCACCTGTATTCCCATCCCATCATCTTGGGCTTTCG

       710        720        730        740        750
CAAAATACCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTA

       760        770        780        790        800
CTAGTGCCGTTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTCTGGCT

       810        820        830        840        850
TTCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAACATCT

       860        870        880        890        900
TGAGTCCCTTTATGCCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATAC

       910        920        930        940        950
ATTTAAACCCTCACAAAACAAAAAGATGGGGATATTCCCTTCAATTCATG

       960        970        980
GGATATGTAATTGGGGGTTGGGGCTCCTTG
```

Figure 37 continued

Figure 38. Patient L Pol

```
         10         20         30         40         50
EDWGPCTEHGEHRIRTPRTPARVTGGVFLVDKNPHNTTESRLVVDFSQFS

         60         70         80         90        100
RGNTRVSWPKFAVPNLQSLTNLLSSNLSWLSLDVSAAFYHLPLHPAAMPH

        110        120        130        140        150
LLVGSSGLSRYVARLSSNSRIINHQHRTMQNLHDSCSRNLYVSLMLLYKT

        160        170        180        190        200
YGRKLHLYSHPIILGFRKIPMGVGLSPFLLAQFTSAVCSVVRRAFPHCLA

        210        220        230        240        250
FSYMDDVVLGAKSVQHLESLYAAVTNFLLSLGIHLNPHKTKRWGYSLQFM

GYVIGGWG
```

Figure 38

Figure 39 Patient L HBsAg

```
        10        20        30        40        50
MENIASGLLGPLLALQAGFFSLTKILTIPQSLDSWWTSLNFLGGTPVCLG

        60        70        80        90       100
QNSQSQISSHSPTCCPPICPGYRWMCLRRFIIFLCILLLCLIFLLVLLDY

       110       120       130       140       150
QGMLPVCPLIPGSSTTSTGPCRTCTTPAQGTSMFPSCCCTKPTDGNCTCI

       160       170       180       190       200
PIPSSWAFAKYLWEWASVRFSWLSLLVPFVQWFVGLSPTVWLSVIWMMWY

       210       220
WGPSLYNILSPFMPLLPIFFCLWVYI
```

Figure 39

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